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Vol. 34 January, 1957 No. 1

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CHAPTER XI. *Milling Begins Moving Westward*

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As the rich land increased its bounty, settlements soon grew in size, and so did the mills.

These self-sufficient small settlements were practically isolated until better roads reached them. There is an instance in Indiana of a thriving small center which was so remote that almost half of the Nineteenth Century had passed before it had more than a semi-annual contact by wagon train with the nearest major trading center, Cincinnati.

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A FLOOD OF GRAIN

As the land filled with settlers a flood of products poured to the Atlantic: the grains, wheat and corn; cattle; coal and ores. The earlier isolated communities tended to disappear. Rural areas were more closely linked with urban centers. New patterns of living emerged and new methods of large scale production were created to meet the new conditions. We shall tell about this development as it affects milling in our next chapter.

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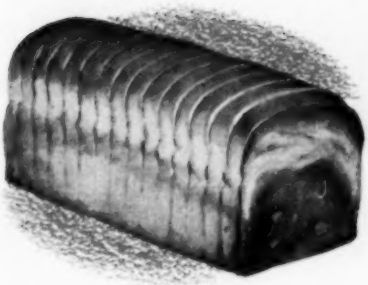

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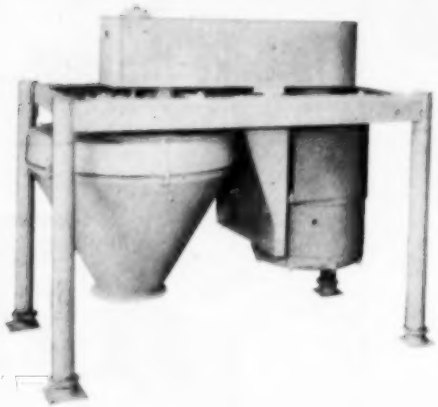

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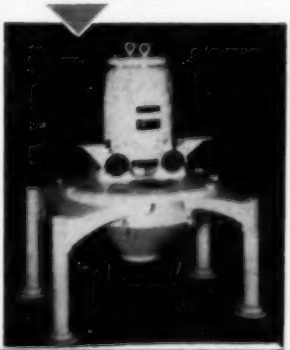

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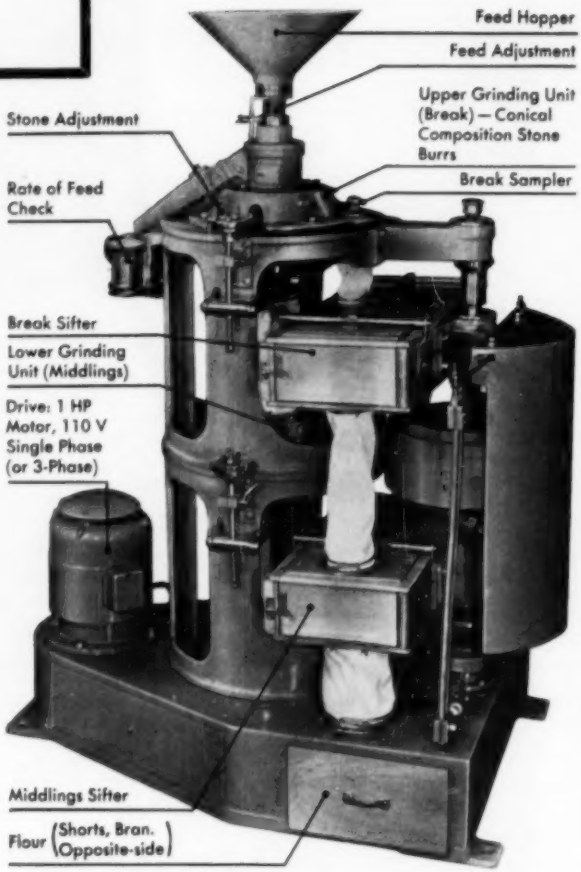

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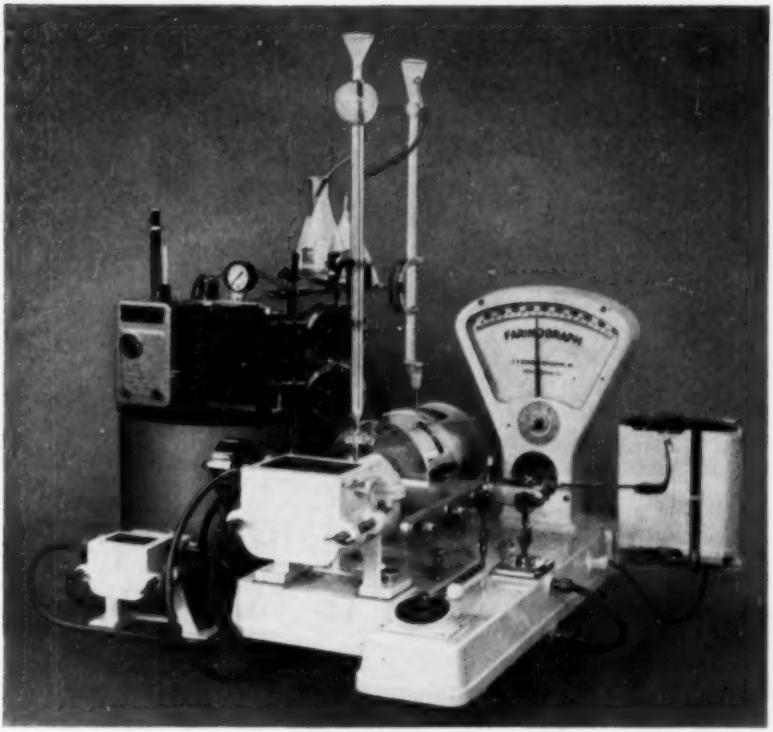

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CEREAL CHEMISTRY

VOL. 34 JANUARY, 1957 NO. 1

THE EFFECT OF COMPLEXING AGENTS ON THE BROMATE REACTION IN DOUGH[1]

I. HLYNKA

ABSTRACT

The rate of bromate reaction in dough was markedly influenced when the level of metal ions was reduced by the addition of complexing agents to dough. Structural relaxation data on doughs containing phytate, oxalate, or ethylenediamine tetraacetic acid (versene) showed that these reagents, when present together with bromate, increased the effect of bromate on the rheological properties of salted and unsalted doughs. When these reagents were present alone they had no appreciable effect on dough properties. Versene was effective in concentrations as low as 40 p.p.m.; to obtain a comparable effect required about 0.1% of phytate or oxalate. The enhanced rheological effect of bromate in doughs containing complexing agents appeared to be paralleled by a greater loss of bromate in doughs after a reaction time of 3 hours. Phytate and versene were also shown to decrease the optimum bromate requirement of flour in the baking test. It is suggested that the role of complexing agents is an indirect one of decreasing the availability of inorganic ions, and that this in some way makes the conditions favorable for an accelerated reaction of bromate. The concentration of inorganic ions and of phytic acid in flour may contribute to the bromate response of flour.

It has been recognized for a long time that mineral constituents are involved in determining dough properties and bread quality. In 1916 Kohman, Hoffman, Godfrey, Ashe, and Blake (12) described the influence on bread of the mineral constituents in the water used. In 1924 Dill and Alsberg (7) established the importance of mineral salts on the properties of gluten in the gluten washing test. Rich (14) showed that the ash content of flour was related to the amount of protein that was peptized in salt solutions. More recently Lusena (13) showed that water-soluble mineral constituents of flour must be extracted in a preliminary step before gluten proteins can be quantitatively solubilized in acetic acid solutions. Walden and McConnell (15) found that they could fractionate and reconstitute flour without damage to baking quality when they used sodium chloride solutions instead of water in their fractionations. In addition to these instances indicating a direct in-

[1] Manuscript received April 30, 1956. Paper No. 157 of the Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg 2, Manitoba, and No. 337 of the Associate Committee on Grain Research (Canada). Presented at the 41st annual meeting, New York, 1956.

volvement of mineral elements in flour quality, the widely accepted practice of using ash as one of the criteria in flour specification should also be noted. The ash content in this instance serves as an indicator of the efficiency of milling and of the rate of extraction and of flour color. It is an index of flour quality by association.

In the present study, evidence is presented for still another role of inorganic ions in relation to flour quality. It is shown that the rate of bromate action in dough is increased very strikingly when complexing or chelating agents are added to bind the metal ions. This paper summarizes the results of studies of the rate of bromate reaction in doughs in which the level of metal ions was reduced by ion-binding reagents.

Materials and Methods

The flour used in this study, except in the baking tests, was milled on a laboratory Buhler mill to an extraction of about 70% from a composite sample of Canadian hard red spring wheat. The protein content of the flour was 12.7%, ash 0.40%, and the absorption used was 64%. For the baking test, a flour was selected that showed a large increase in loaf volume on the addition of bromate. The flour was a clear that had been used in another study (4). It had a protein content of 15.2%, ash 0.92%, and an absorption of 62.9%. All the data are on a 14% moisture basis.

Doughs for experiments other than baking were prepared by mixing 100 g. flour with water, and solutions of salt, potassium bromate, ammonium oxalate, sodium phytate (pH 5.8), and ethylenediamine tetraacetic acid (versene) as required for each specific experiment. Doughs were mixed for 2.5 minutes in an atmosphere of nitrogen in a G.R.L. dough mixer (10) with temperature control to give doughs at $30 \pm 0.5°C$.

Changes in the properties of doughs thus prepared were followed by the method of structural relaxation already described (5, 6, 9). Briefly, doughs were given 5 minutes, 1, 2, 4, or 6 hours' reaction time to bring out the effect of bromate on dough properties. At the end of the desired reaction time doughs were shaped on the extensograph and secured on dough holders. Extensograms were then obtained on successive doughs after rest periods of 5, 10, 25, 45, 75, and 105 minutes. The extensogram loads were then read at an extension of 5 cm. on the chart for unsalted doughs and at 7 cm. for salted doughs, and structural relaxation curves were obtained by plotting these loads against rest period.

Two constants characterizing dough properties were evaluated from the structural relaxation curves. These have been described in previous

publications (5, 9). The first of these constants is the asymptotic load L_A and is the load which the relaxation curve will theoretically approach at infinitely long rest periods. This constant is obtained as the slope of the linear transformation of the relaxation curve. A second constant, C, called the relaxation constant, is related to the curvature of the relaxation curve. It is obtained as the intercept of the linear transformation. Both these constants are required to completely characterize the relaxation curve. They were evaluated from the linear transformation of the relaxation data by the method of least squares.

Doughs were analyzed for bromate content after various reaction times by the method of Cunningham and Anderson (2, 3). This method involved preparing a zinc sulfate-sodium hydroxide extract of dough, adding sulfuric acid, potassium iodide, and excess thiosulfate, and back-titrating with standard iodate. The end point was determined amperometrically.

The baking tests employed the basic A.A.C.C. malt phosphate formula. However, the yeast concentration was reduced to 2% in order to obtain a large loaf volume increase at the optimum level of bromate.

Results

The primary object of the experiments described in this section was to study the rate of bromate reaction in dough to which phytate, oxalate, and versene (ethylenediamine tetraacetic acid) were added to complex the inorganic ions, presumably Ca^{++} and Mg^{++}, in the dough. In order to ensure minimum interference from other ions, one part of the experiments was done with doughs to which no salt was added. A parallel set of experiments was then done with doughs containing 1% salt (flour basis) as is customary in dough testing. The results from two subsidiary experiments, one on the decomposition of bromate in doughs containing complexing agents, and one on the optimum requirement of bromate in the baking test with these reagents, will conclude the presentation of data.

The Action of Bromate in Unsalted Doughs Containing Phytate, Oxalate, and Versene. Structural relaxation curves were obtained for doughs containing 20 p.p.m. bromate together with 0.1% sodium phytate at pH 5.8; 0.2% ammonium oxalate and 40 p.p.m. versene (all on flour basis). Control experiments were also done with doughs containing these reagents only. Reaction times of 5 minutes, 1, 2, 4, and 6 hours were given. It should be noted here that the extensograms obtained with unsalted doughs were much smaller than those ordinarily obtained with salted doughs. For this reason, 5 cm. was selected as the extension for obtaining the loads from extensograms.

The results for unsalted doughs are summarized in Fig. 1 as the linear transformations of structural relaxation curves where the rest period is plotted against the product of the load and the rest period. In this form the effect of potassium bromate and of the complexing agents can be seen at a glance by comparing the intercepts and the slopes of the curves.

The graphs A to D on the left-hand side of Fig. 1 show the control experiments with doughs containing no bromate. Doughs in A contained flour and water only, doughs contained in addition 0.1% phytate in B, 0.2% oxalate in C, and 40 p.p.m. versene in D. The results in all the control experiments are closely similar to the results on doughs containing water only. They indicate that these reagents when present in the dough alone have essentially no effect. Oxalate is a possible exception, since it gave curves that were very slightly higher; it is likely, however, that at this concentration oxalate may exhibit a small salt effect. This was also indicated by the observation that extensograms for doughs containing oxalate tended to be larger. In all the control experiments the slopes of the lines for doughs given shortest reaction times were greatest and decreased progressively as the reaction time increased.

The right-hand side of Fig. 1 summarizes the effect of the complexing agents on the reaction of bromate. All the doughs shown in graphs E to H contained 20 p.p.m. bromate. The doughs in E contained bromate only, those in F contained in addition 0.1% phytate, 0.2% oxalate in G, and 40 p.p.m. versene in H. The data in the right half of Fig. 1 are thus entirely parallel to those in the left half except that all doughs contained 20 p.p.m. bromate. A comparison of graph E for doughs containing bromate only with F, G, and H which contained bromate plus the complexing agents shows that the presence of these agents markedly increases the slopes of the curves. In other words, complexing the inorganic ions accelerates the reaction of bromate in dough, especially, of course, at the longer reaction times. It required approximately twice as much oxalate as phytate to produce an equal effect; versene produced the most striking acceleration of the bromate reaction.

Figure 2 shows a plot of the asymptotic load L_A and the relaxation constant C against reaction time for the data that were presented in Fig. 1. The change of asymptotic load with reaction time is shown in the upper part of Fig. 2. The curves for doughs containing phytate plus bromate and oxalate plus bromate are very similar to one another, although the concentration of oxalate on a weight basis was twice that of phytate. The curve for doughs containing bromate plus versene is

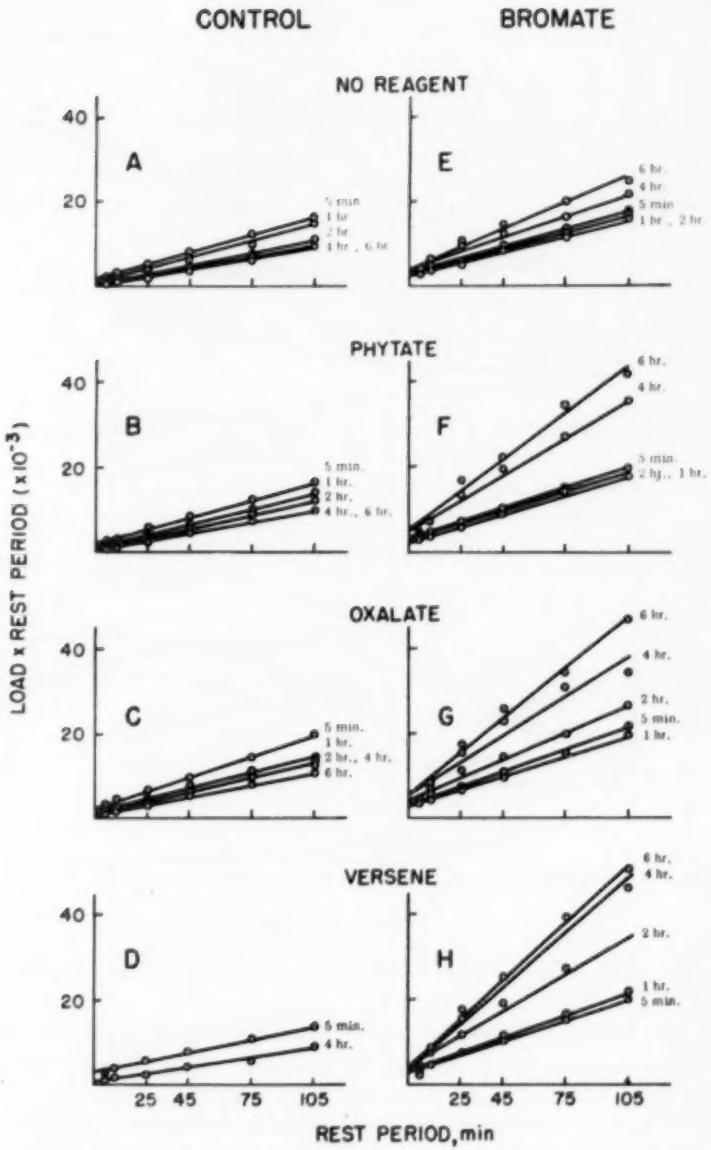

Fig. 1. Linear transformations of structural relaxation curves showing the effect of phytate, oxalate, and versene on reaction of bromate in unsalted doughs for reaction times of 5 minutes, 1, 2, 4, and 6 hours. Graphs A to D show data on dough containing: no reagents; 0.1% phytate; 0.2% oxalate; and 40 p.p.m. versene. Graphs E to H show data on identical doughs but containing in addition 20 p.p.m. bromate.

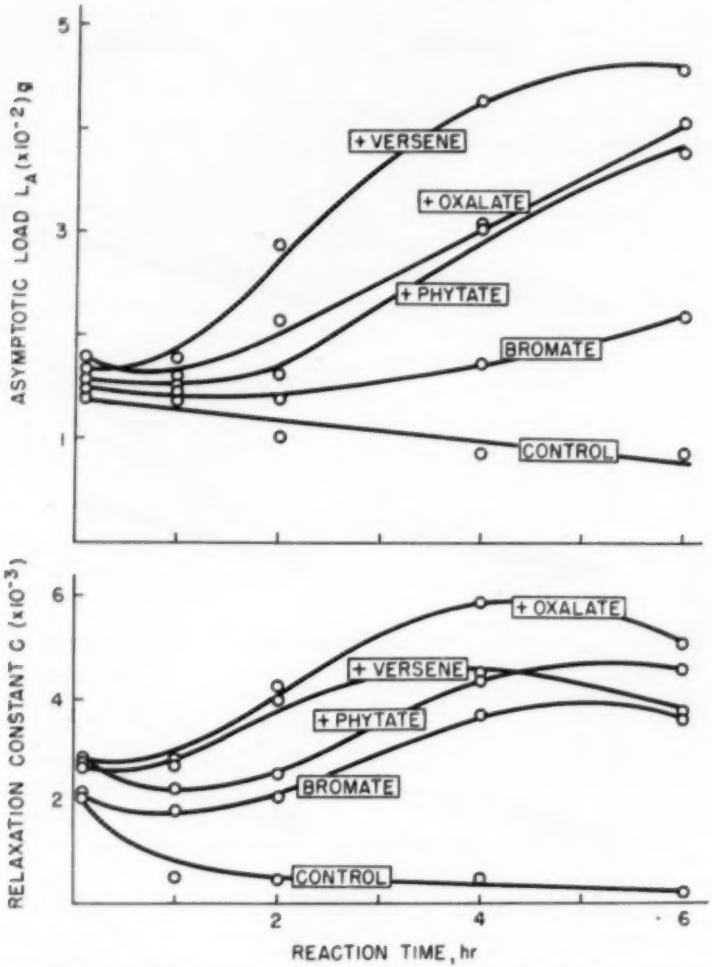

Fig. 2. Change in asymptotic load L_A (above) and of relaxation constant C (below) with reaction time. Dough treatment: unsalted doughs containing no reagents; 26 p.p.m. bromate; and bromate plus phytate, oxalate, or versene.

not only higher but also shows a leveling off at the long reaction time.

In comparison the curves for the change of the relaxation constant with reaction time, in the lower part of Fig. 2, show some interesting contrasts. There appears to be a characteristic dip in most of the curves in the first hour. All curves, except that for control doughs with water only, are S-shaped. The curve for the doughs containing bromate plus oxalate is higher than the curves containing bromate plus phytate, and

bromate plus versene. In other respects the data obtained with the asymptotic load L_A are confirmed by those obtained with the relaxation constant C.

Phytic acid is a normal constituent of flour and is also a complexing agent. For white flour, Kent-Jones and Amos (11) give a range of 0.025–0.05% and a somewhat lower value of 0.015% from McCance and Widdowson. These values are in terms of phytic acid phosphorus; in terms of phytic acid they would be somewhat higher. It was there-

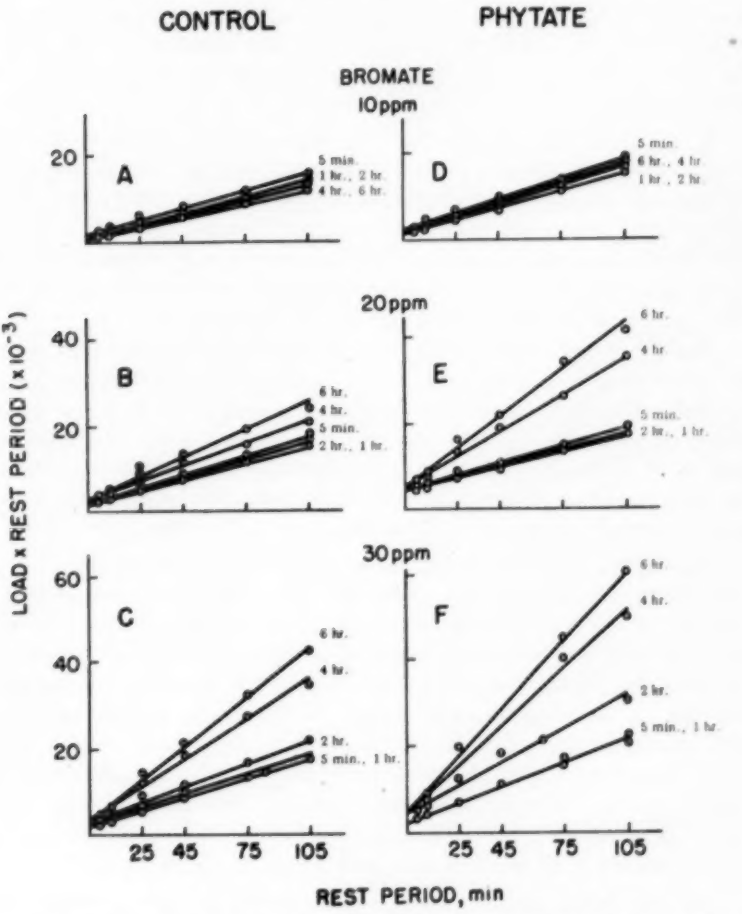

Fig. 3. Linear transformations of structural relaxation curves showing the effect of phytate at different levels of bromate in unsalted doughs at reaction times of 5 minutes, 1, 2, 4, and 6 hours. Graphs A to C show data on doughs containing 10, 20, and 30 p.p.m. bromate. Graphs D to F show data on doughs containing 0.1% phytate at these bromate levels.

fore considered of some interest to include a few additional experiments with phytic acid.

Additional data on the effect of 0.1% phytate on doughs containing 10, 20, and 30 p.p.m. bromate are presented in Fig. 3. The data are in terms of linear transformations of structural relaxation curves. The graphs A, B, and C show the effect of 10, 20, and 30 p.p.m. bromate present alone given reaction times of 5 minutes, 1, 2, 4, and 6 hours as before. Graphs D, E, and F in the right-hand half of Fig. 3 show the data on doughs containing 10, 20, and 30 p.p.m. bromate together with 0.1% phytate. The effect of phytate is small but definite in D and pronounced in E and F. The effect of 30 p.p.m. bromate in C is about the same as that in F for doughs containing 20 p.p.m. bromate plus 0.1% phytate.

The Action of Bromate in Salted Doughs Containing Phytate, Oxalate, and Versene. Because salt is commonly used in doughs prepared for the extensograph test, and in the baking test, the effect of bromate in presence of complexing agents in salted doughs was also studied. The results on salted doughs are summarized in Fig. 4 in the form of linear transformations of structural relaxation curves as before. Loads were read at an extension of 7 cm. on the extensograms.

The data summarized in Fig. 4 are arranged in the same way as the previous data on unsalted doughs. The graphs on the left-hand side are the control experiments on doughs containing 1% salt. A contained water only, B contained in addition 0.1% phytate, C 0.1% oxalate, and D 40 p.p.m. versene. Again, visual inspection of the data shows that none of these reagents in salted doughs has any appreciable influence on dough properties in themselves.

The right-hand part of Fig. 4 shows the effect of 20 p.p.m. of bromate in E. The doughs in F contained 20 p.p.m. bromate plus 0.1% phytate, in G the doughs contained 20 p.p.m. bromate plus 0.1% oxalate, and in H only 10 p.p.m. bromate and 40 p.p.m. versene. Just as in unsalted doughs, the presence of complexing agents markedly increased the effect of bromate in salted doughs. It should be pointed out that the concentration of oxalate was reduced to 0.1% as compared with 0.2% in unsalted doughs. In presence of 1% salt, the salt effect of oxalate was not perceptible in the control experiment (graph C). The effect of versene was so pronounced that bromate concentration in doughs shown in H was reduced from 20 p.p.m. to 10 p.p.m.

The foregoing data are more clearly shown in Fig. 5, where the asymptotic load and the relaxation constant are plotted against reaction time. The upper half of Fig. 5 shows the influence of complexing

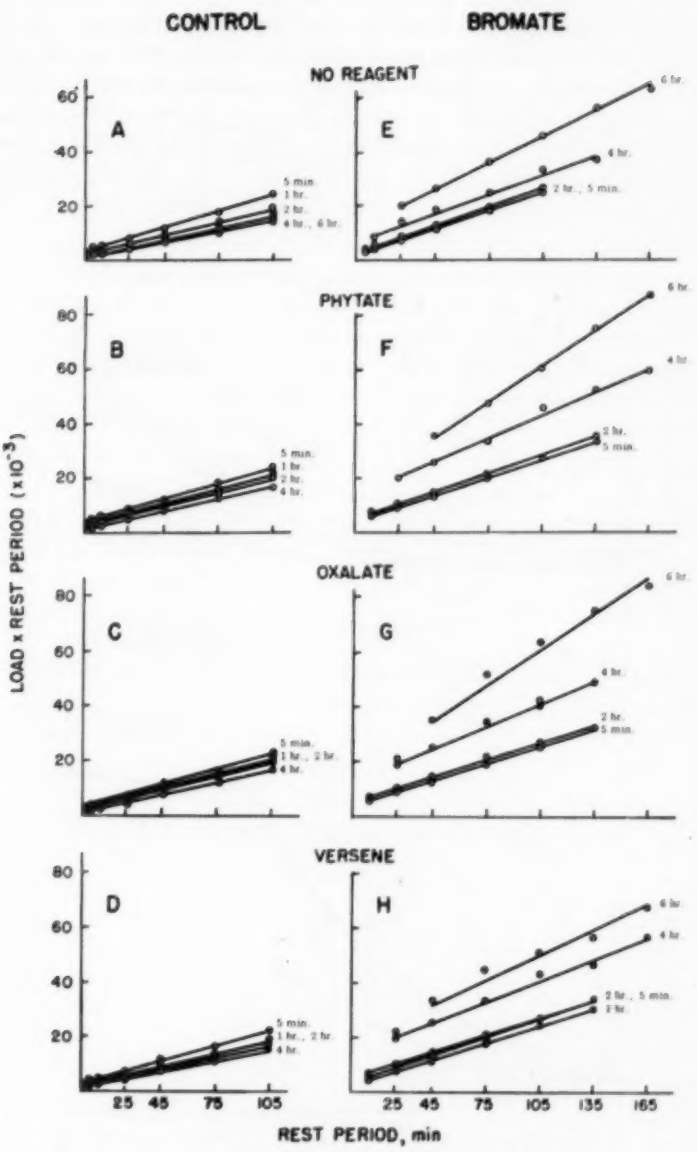

Fig. 4. Linear transformations of structural relaxation curves showing the effect of phytate, oxalate, and versene on the action of bromate in salted (1%) doughs for reaction times of 5 minutes, 1, 2, 4, and 6 hours. Graphs A to D show data on dough containing: no reagents; 0.1% phytate; 0.1% oxalate; and 40 p.p.m. versene. Graphs E to H show data on identical doughs but all containing in addition 20 p.p.m. bromate, except in H where bromate concentration was reduced to 10 p.p.m.

agents on the reaction of bromate in dough with time as indicated by changes in the asymptotic load L_A. The effect of phytate and oxalate are very similar. The curve for doughs containing bromate plus versene is below those for phytate and oxalate, but it should be remembered that only 10 p.p.m. instead of 20 p.p.m. bromate were used in the versene experiments to confine the extensograms to workable limits. The lower half of Fig. 5 shows the influence of the complexing agents on the variation of relaxation constant with reaction time in salted doughs.

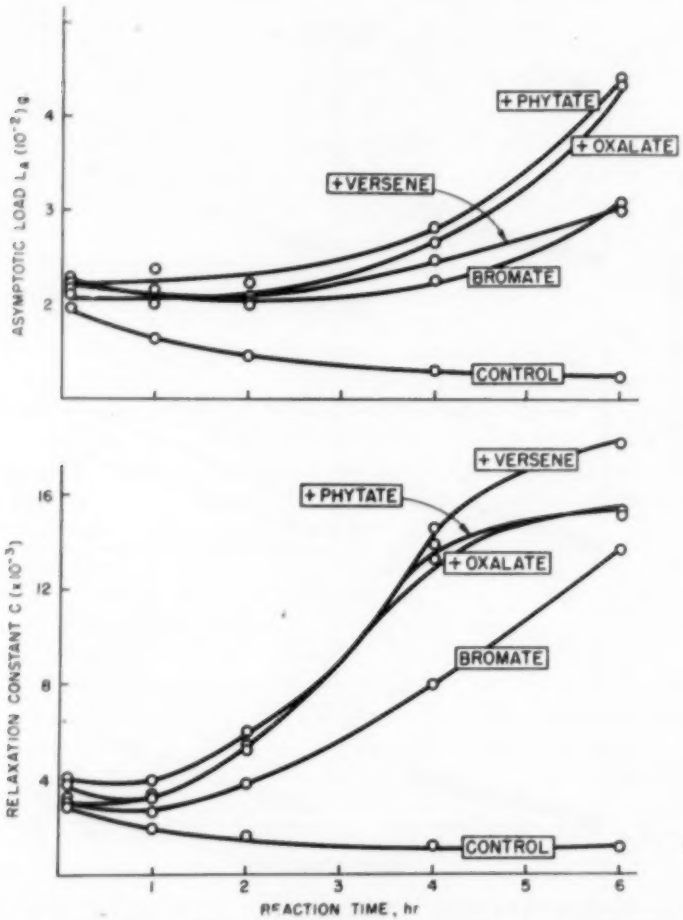

Fig. 5. Change in asymptotic load L_A (above) and relaxation constant C (below) with reaction time. Dough treatment: salted doughs containing no reagents; 20 p.p.m. bromate; 20 p.p.m. bromate plus 0.1% phytate, or 0.1% oxalate; and 10 p.p.m. bromate plus 40 p.p.m. versene.

The data for all three conditions, 20 p.p.m. bromate plus 0.1% phytate, 20 p.p.m. bromate plus 0.1% oxalate, and 10 p.p.m. bromate plus 40 p.p.m. versene, are very similar. There is a leveling off of the curves at higher reaction times.

Decomposition of Bromate in Doughs Containing Phytate, Oxalate, and Versene. The data presented so far indicate that phytate, oxalate, and versene increase the effect of bromate in dough, as indicated by changes in physical dough properties. Preliminary experiments were done to determine whether analogous chemical changes could be detected. Accordingly doughs were prepared to contain bromate, bromate plus phytate, bromate plus oxalate, and bromate plus versene. The salted and unsalted doughs were analyzed by the method of Cunningham and Anderson (2, 3) at reaction times of zero and 3 hours at 30°C. to determine the amount of bromate remaining. Three hours' reaction time was selected to correspond with the time the loaf of bread is molded in the baking test.

The analytical data are summarized in Table I and are expressed in p.p.m. bromate found at the end of the specified reaction time. The data on doughs given zero reaction time showed very satisfactory recoveries. After 3 hours' reaction time both salted and unsalted doughs containing bromate and versene gave the lowest recoveries. A slightly higher recovery was obtained with doughs containing bromate plus oxalate. Doughs with bromate plus phytate gave recoveries nearly the same as doughs containing bromate only; the result for the salted phytate dough seemed to be lower.

TABLE I

BROMATE RECOVERIES IN SALTED AND UNSALTED DOUGHS CONTAINING BROMATE ONLY, AND BROMATE PLUS PHYTATE, OXALATE, OR VERSENE

TREATMENT	REACTION TIME	BROMATE RECOVERY	
		Salt, 0%	Salt, 1%
	hr.	p.p.m.	p.p.m.
Bromate, 20 p.p.m.	0	20.4	20.1
	3	15.5	16.7
$KBrO_3$, 20 p.p.m. + sodium phytate, 0.2%	0	19.8	20.2
	3	15.7	16.1
$KBrO_3$, 20 p.p.m. + oxalate, 0.2%	0	19.6	19.8
	3	15.0	15.7
$KBrO_3$, 20 p.p.m. + versene, 40 p.p.m.	0	20.0	...
	3	14.5	14.2

The enhanced rheological effect of bromate-treated doughs containing complexing agents thus appears to be paralleled by a greater

bromate loss in the dough. On the whole, the analytical data are considered as suggestive rather than conclusive.

Effect of Phytate and Versene on the Optimum Bromate Requirement in the Baking Test. If it is tentatively accepted that the rheological and analytical data that have been presented indicate that bromate reacts more rapidly in doughs containing complexing agents, then it may be reasonable to expect that a smaller amount of bromate in presence of these agents would be required to produce a given effect than if bromate alone were used. This hypothesis was tested by means of baking experiments.

The flour selected for the baking experiments had an optimum bromate requirement of 25 p.p.m., as was established previously by data obtained in a series of loaves baked with a range of bromate concentrations from 0 to 40 p.p.m. In the present study the range of bromate concentrations used was 0 to 25 p.p.m. in 5-p.p.m. increments. Three series of loaves were baked. The first series contained bromate alone, the second contained bromate plus 0.1% phytate, and the third series contained bromate plus 20 p.p.m. versene. The results are summarized in Fig. 6.

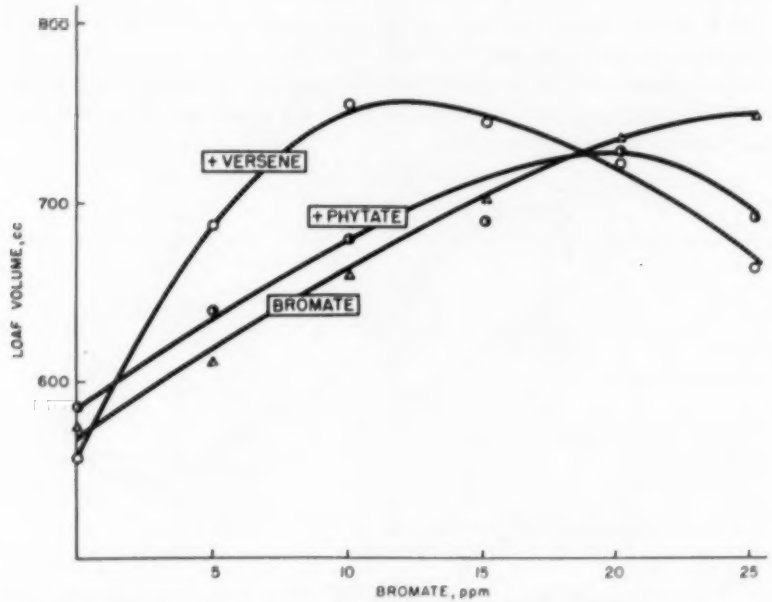

Fig. 6. Effect of phytate and versene on the optimum bromate requirement in the baking test.

The curve for the first series of loaves with bromate alone provides a basis of reference, with an optimum bromate requirement of 25 p.p.m. The curve for the second series of loaves containing bromate plus phytate shows an optimum bromate requirement of about 20 p.p.m. The curve for the third series of loaves containing bromate plus versene shows an optimum loaf volume of between 10 and 15 p.p.m. of bromate. It is also of interest to note that the maximum loaf volumes in the three series of baking trials were 750, 730, and 755 cc. Differences of this magnitude are generally considered to be within the error of the test-baking procedure. The complexing agents thus did not appear to have deleterious effects; they simply reduced the optimum bromate requirement.

Discussion

The findings that phytate, oxalate, and versene accelerate the reaction of bromate in dough raise two important questions. The first of these relates to the mechanism of the acceleration reaction and the second to the probable role of phytate on the one hand, and of inorganic ions on the other, in the response of flour to bromate.

The mechanism of the apparent catalytic activity of phytate, oxalate, and versene can only be inferred from circumstantial evidence. None of these reagents showed an appreciable effect on dough properties when present alone. Moreover, they are not readily oxidizable and reducible and would therefore not be expected to possess catalytic activity for the bromate reaction in dough similar to that shown, for example, by vanadium salts. The property that is common to phytate, oxalate, and versene is their pronounced complexing affinity, especially for calcium as well as for magnesium and other bidentate ions. This complexing property suggests that the bidentate inorganic ions normally present in dough in some way inhibit the reaction of bromate. If this is true, complexing these ions with chelating agents makes it possible for the bromate reaction in dough to proceed more rapidly. The influence of inorganic ions thus appears to be an indirect one.

The availability of calcium, magnesium, etc. in flour and dough will depend not only on the total concentrations of these ions but also on the concentration of complexing agents naturally present. Kent-Jones and Amos (11) and Bailey (1) have summarized much of the key literature on phytic acid and on the mineral ions present in wheat and wheat products. Phytic acid is present in small amounts in white flour and increases in the longer-extraction flours. It may constitute an important part of the phosphorus in flour ash. Phytic acid in flour may be present as a calcium-magnesium salt. Wheat flour also contains an

enzyme phytase which hydrolyzes phytic acid to inositol. As much as 80% phytic acid may be decomposed during dough fermentation of a good-grade flour. Thus the availability of calcium and magnesium ions may increase, and the bromate reaction may be correspondingly decreased in fermenting dough. Whether this may be associated with the leveling off in the effect of bromate as shown in Figs. 2 and 5 is an interesting possibility. In addition to phytic acid, it is likely that wheat proteins and perhaps other lesser constituents may also act as complexing agents.

The availability of calcium in the presence of phytic acid has been studied from a nutritional point of view and is summarized by Kent-Jones and Amos (11). This however, may be somewhat different from the specific role of calcium in relation to the bromate reaction. The availability of magnesium has been of less interest, although magnesium is present in more than twice the concentration of calcium. Recently El-Gindy, Burell, and Lamb (8) have provided data on the distribution of inorganic ions in the gluten, starch, and water-soluble fractions in flour. Also Cunningham and Anderson (3) observed a positive correlation between the total ash (a good portion of which may arise from phytic acid phosphorus), and the rate of bromate disappearance in dough for a series of flours.

Although emphasis has been placed on calcium and magnesium ions, there is a possibility that other ions such as iron and copper may be involved. These ions are known to combine with sulfhydryl groups. If the bromate reaction involves the sulfhydryl group, then complexing the heavy metal ions should accelerate the reaction.

The response of a given flour to bromate thus appears to be influenced in a rather complex manner by various flour constituents. The present study has served to draw attention to the necessity of reexamining the role of inorganic ions on the one hand and of complexing agents on the other in their relation to the response of flour to bromate. It is also hoped that further study in this area will contribute, although perhaps indirectly, to the eventual elucidation of the mechanism of the improver action of bromate in dough.

Acknowledgment

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FURTHER DEVELOPMENTS IN THE SEDIMENTATION TEST FOR WHEAT QUALITY[1]

A. J. Pinckney[2], W. T. Greenaway[2], and Lawrence Zeleny[3]

ABSTRACT

The sedimentation test has been modified to make it applicable to soft as well as hard wheat, and to provide greater uniformity of interlaboratory results. A simple milling procedure has been developed for preparing small samples suitable for the test.

Highly significant correlations have been obtained consistently between sedimentation values and bread loaf volume. Since the sedimentation test reflects differences in both protein content and gluten quality, it is particularly useful for wheat evaluation when large differences in gluten quality are prevalent. When gluten quality is uniform, the sedimentation test offers only the advantages of speed and simplicity over the Kjeldahl protein test.

The sedimentation test appears to be slightly more reliable than the farinograph test as an index of over-all baking strength; but, unlike the farinograph test, it does not measure specific factors such as mixing time, mixing tolerance, and water absorption.

Satisfactory interlaboratory agreement has been obtained by operators not trained as chemists.

Because of its speed and simplicity, the sedimentation test should prove useful in routine wheat inspection procedures. A classification of wheat according to baking strength as measured by the sedimentation test is suggested.

The sedimentation test which was described in an earlier paper (5) gives results that depend largely on the quantity and quality of the gluten in experimentally milled wheat flour. Highly significant correlations were found between sedimentation values and bread-loaf volumes. Inferior gluten quality, as shown by loaf volumes significantly less than the values predicted from the protein content, was generally reflected by correspondingly low sedimentation values.

The test as originally described had some serious limitations. No simple method was available for preparing suitable test flour from small samples of wheat. The test sometimes failed, especially when applied to soft-wheat flours. Interlaboratory agreement was often unsatisfactory. Research was therefore continued to make the test more useful for the evaluation of wheat and to study further the relation between sedimentation value and baking strength. The factors influencing the volume of the sediment have been studied. Various modifications of the test have been tried. The statistical relationships were found to be nearly independent of the modification used. Of the modifications studied only two were of practical importance. Both involved the use of isopropyl alcohol and a reduction of the lactic acid concentration.

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[2] Chemists, Field Crops Research Branch, Agricultural Research Service.

[3] Chief, Standardization and Testing Branch, Grain Division, Agricultural Marketing Service.

The test herein described provides a simple method for the preparation of test flour, gives sharp readings in testing all classes of wheat, and furnishes normally satisfactory interlaboratory agreement. In addition, it retains the advantages of the original test. Since the test is highly empirical, it is necessary to adhere closely to all details of the procedure.

Method I – for Wheat

Equipment: (1) Motor-driven, corrugated steel rolls equivalent to Tag-Heppenstall moisture meter rolls for small grains.

(2) Sieve, 100-mesh (U. S. Standard No. 100 woven-wire cloth sieve), 8 inches in diameter, equipped with bottom pan.

(3) Glass-stoppered 100-ml. graduated cylinders having a distance of from 180 to 185 mm. between the zero mark at the bottom and the 100-ml. mark at the top.

(4) Stop-watch or interval timer.

(5) Mixer (optional). A rack approximately 23 by 12 by 2 inches is pivoted at the center of each end and oscillated through 60°, 30° each side of the horizontal position, at the rate of about 40 times per minute. The rack is so designed that eight graduated cylinders may be quickly and securely placed while the mixer is in motion. Power is supplied by a small electric motor.

Reagents: (1) Isopropyl alcohol, 99–100%, N.F., or equivalent.

(2) Distilled water containing 4 mg. of brom phenol blue per liter.

(3) Lactic acid stock solution. Dilute 250 ml. of U.S.P. 85% lactic acid to 1 liter with distilled water. Reflux the diluted acid 6 hours without loss of volume (see Note 1).

(4) Mix thoroughly the following:

180 ml. of lactic acid stock solution (reagent 3)
200 ml. of isopropyl alcohol, 99–100%
Distilled water to make 1 liter

Let the reagent stand 48 hours before using. Protect against evaporation.

Procedure: (1) Grind about 200 g. of wheat by passing it five times through the motor-driven rolls adjusted to a clearance of 0.023 inch and turning at about 30 r.p.m. If Tag-Heppenstall moisture meter rolls are used, this clearance is attained by using the flaxseed shims (see Note 2).

(2) Place the ground wheat on the 100-mesh sieve equipped with bottom pan and shake by hand or mechanically for 1½ minutes. The shaking should be done with a horizontal circular motion in such a way that any point on the sieve will describe circles about 2 inches

in diameter at the rate of about 200 per minute. If the shaking is done by hand, set the sieve assembly on a smooth level surface and move as described. The yield should be about 25 g.

Be *certain* the sieve is *clean* before using. The flour must be thoroughly mixed before testing.

(3) Place 3.2 g. of the flour to be tested in a 100-ml. glass-stoppered graduated cylinder.

(4) Simultaneously, start the timing and add 50 ml. of distilled water containing brom phenol blue. Mix thoroughly the flour and water by moving the stoppered cylinder horizontally lengthwise, alternately right and left through a space of 7 inches, twelve times in each direction in 5 seconds. The flour should be completely swept into suspension during the mixing.

Proceed with steps 5, 6, and 7 or 5a, 6a, and 7a.

Mechanical Mixing

(Use only a mixer that will simulate the motion described in item 5 under equipment)

(5) Place the cylinder in the mixer and mix for 5 minutes.

Hand Mixing

(5a) At the end of the first 2-minute period, mix the contents for 30 seconds in this manner: Completely invert, then right the cylinder, as if it were pivoted at the center (see Fig. 1). Perform this action smoothly, exactly 18 times in the 30 seconds. Let it stand 1 minute and 30 seconds.

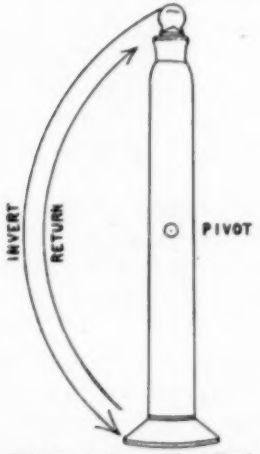

Fig. 1. Mixing action as described in 5a.

(6) Remove from the mixer, add 25 ml. of the isopropyl alcohol-lactic acid reagent and mix in the mixer for 5 minutes.

(6a) Add 25 ml. of the isopropyl alcohol-lactic acid reagent, mix immediately by inverting the cylinder four times, as in 5a. Let stand 1 minute and 45 seconds.

(7) Remove from the mixer. *Immediately* place the cylinder in an upright position and let stand for *exactly* 5 minutes.

(7a) Mix for 30 seconds as in 5a, let stand 1 minute and 30 seconds. Again mix for 15 seconds and *immediately* place the cylinder in an upright position and let stand for *exactly* 5 minutes.

(8) At the end of 5 minutes, read the volume in ml. (estimating tenths of a ml.) of the sediment in the cylinder. This is the uncorrected sedimentation value.

(9) To obtain the corrected sedimentation value (14% moisture basis), multiply the uncorrected sedimentation value by the appropriate factor in the following table:[4]

Wheat Moisture	*Factor*	*Wheat Moisture*	*Factor*	*Wheat Moisture*	*Factor*
%		%		%	
8.0	1.14	11.0	1.00	14.0	1.00
8.5	1.10	11.5	0.99	14.5	1.02
9.0	1.07	12.0	0.98	15.0	1.04
9.5	1.05	12.5	0.98	15.5	1.07
10.0	1.03	13.0	0.98	16.0	1.10
10.5	1.01	13.5	0.99		

NOTES: (1) Concentrated lactic acid normally contains associated molecules which on dilution gradually dissociate in part until a state of equilibrium is reached. Attainment of equilibrium after dilution, which is necessary for consistent sedimentation test results, is greatly hastened by refluxing. The refluxed stock solution should be about 2.78 normal. The mixed reagent is approximately 0.5 normal and contains 20% of isopropyl alcohol.

(2) The wheat used for preparing the flour may be that upon which a moisture test has been made with the Tag-Heppenstall moisture meter.

Method II – for Flour

Proceed as for wheat, omitting steps 1 and 2. The factors given in paragraph 9 are not to be applied in testing flour. To convert the un-

[4] The moisture content of wheat when ground as above partly determines the content of ash and of protein in the resultant flour. Ash and protein, in turn, influence the sedimentation result. The factors in the table above are designed to compensate for variations in moisture content and to correct the sedimentation values to the 14% moisture basis.

corrected flour sedimentation value to the 14% moisture basis, use the formula:

$$\text{Sedimentation value, corrected to 14\% moisture basis} = \text{sedimentation value, uncorrected} \times \frac{100 - 14}{100 - \text{percent flour moisture}}$$

NOTE: In applying the sedimentation test to flour, it should be used only for comparing different lots of the same grade of flour milled by the same experimental or commercial mill.

Materials and Procedures

Samples representing about 5000 lots of commercial wheat of the crop years 1947–1955, inclusive, were obtained through the field offices of the Grain Division. The samples were selected to represent as wide a range in quality as possible and to represent widely scattered areas of production. In addition, about 1000 variety samples of the hard red spring class, tested in connection with the wheat-breeding program of the Agricultural Research Service, were included.

The percentages of "dark, hard, and vitreous" kernels in the samples of hard red winter and hard red spring wheat were determined by grain inspection supervisors. These data are of interest since they represent the only significant measure of baking strength provided in the U. S. wheat standards.

The wheats were milled to 90-percent patent flours on Allis-Chalmers or Buhler[5] experimental flour mills. The bread-baking tests were made by a formula described by Fifield *et al.* (1) using 100 g. of flour, 2.0 g. of compressed yeast, 1.5 g. of salt, 5.0 g. of sugar, 0.25 g. of malted wheat flour, 3.0 g. of shortening, 4.0 g. of nonfat milk solids, and varying amounts (0 to 4 mg.) of potassium bromate. The ingredients for two loaves were mixed together for a sufficient length of time for proper dough development by using a Hobart-Swanson dough mixer with four pins in the head and two pins in the bowl and operated at 108 r.p.m. The doughs, after mixing, were divided into two equal parts, fermented for 3 hours at 30°C., panned and proofed for 55 minutes at 30°C., then baked for 25 minutes at 232°C. For the purposes of this study the loaf volume data in each instance are that of the loaf containing the amount of potassium bromate that produced the greatest loaf volume. In most instances the loaf having the greatest volume also had the best grain, texture, and crumb color.

The close relationship between protein content and loaf volume of bread baked by procedures like the one described above is generally

[5] The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over those of other firms or similar products not mentioned.

recognized (2, 3). Accordingly, protein content, as determined by the usual Kjeldahl procedure, has been included in this study. Since farinograph curves are commonly used to indicate baking strength, some farinograph results have also been included.

Through a contract with a leading commercial laboratory, 534 samples of hard wheat of the 1947 crop were milled and baked and given farinograph tests. Sedimentation tests were applied to replicate portions of these samples in the laboratory of the authors. The recorded farinograph curves were judged subjectively by the commercial laboratory and were assigned adjective ratings intended to reflect baking strength.

The same commercial cereal laboratory made farinograph curves on 829 samples of wheat of the 1948 crop and evaluated the curves in terms of valorimeter values. Valorimeter values, like sedimentation values, are affected by both gluten quantity and quality, and therefore serve as objective indices of baking strength.

Results

In Table I are shown some statistical values concerning the intercorrelations of sedimentation and protein values; dark, hard, and vitreous kernel percentages; and bread loaf volumes for 6061 samples of wheat of the crop years 1947–1955, inclusive. The ratings of 534 wheat samples of the 1947 crop by farinograph and sedimentation tests are given in Table II, and correlation coefficients and standard errors of estimate are given in Table III for the correlations of loaf volume with valorimeter and sedimentation values, respectively, for 829 samples of wheat, 1948 crop.

Discussion

From the data in Table I it is apparent that sedimentation values and protein content ordinarily reflect bread-baking strength about equally well. In some circumstances, however, sedimentation value provides a significantly better measure of baking strength than does the protein content. This is particularly noticeable in the data for the 1952 and 1953 crops of hard red winter wheat and can be explained by the fact that those two crops contained much wheat of inferior gluten quality, presumably largely as a result of unfavorable weather conditions prior to harvest. Variations in gluten quality are independent of protein quantity but apparently affect bread loaf volume and sedimentation values in much the same way. Consequently, loaf volume is likely to be more closely correlated with sedimentation value than with protein content when the gluten quality is quite variable. This

TABLE I

CORRELATION COEFFICIENTS AND STANDARD ERRORS OF ESTIMATE FOR 6061 SAMPLES OF WHEAT FOR CROP YEARS 1947 THROUGH 1955

(N = number; r = correlation coefficient; S = standard error of estimate)

Crop Year	Class	N	Sedimentation Value (s), Loaf Volume (v), and Protein Content (p)			Protein (p) and Loaf Volume (v)		Sedimentation Value (s) and Protein (p)		Dark, Hard, and Vitreous Kernels (d) and Loaf Volume (v)	
			r_{sv}	S_{vs}	$r_{sv.p}$	r_{pv}	S_{vp}	r_{sp}	S_{ps}	r_{dv}	S_{vd}
				ml.			*ml.*		%		*ml.*
1947	HRW[a]	535[b]	0.59**	83	0.15**	0.62**	80	0.85**	0.8	...	...
	HRS[a]	560[b]	0.83**	43	0.09*	0.84**	42	0.91**	0.6	...	...
	HRW[a]	511[b]	0.65**	54	0.43**	0.57**	58	0.69**	1.0	...	...
	HRS[a]	700[c]	0.86**	65	0.80**	0.89**	58	0.66**	1.6	...	...
	HRW[a]	79	0.94**	39	0.14	0.98**	25	0.95**	0.6	...	...
1948	HRW	513	0.68**	55	0.24**	0.81**	44	0.72**	0.9	0.41**	68
	HRS	265	0.67**	53	0.41**	0.79**	43	0.59**	0.9	0.37**	66
	White	51	0.80**	32	0.52**	0.81**	30	0.73**	0.2	...	...
	Comb.[d]	829	0.79**	55	0.35**	0.86**	45	0.79**	0.9	...	...
	HRS[a]	677[c]	0.75**	64	0.54**	0.78**	61	0.62**	1.3	0.67**	72
1949	HRW	160	0.72**	51	0.58**	0.85**	38	0.56**	1.2	0.56**	56
	HRS	50	0.83**	42	0.82**	0.74**	50	0.70**	0.8	0.64**	57
	SRW	56	0.65**	61	0.42**	0.68**	59	0.59**	0.9	...	...
	White	256	0.78**	70	0.44**	0.85**	60	0.74**	1.3	...	...
	Comb.[d]	522	0.83**	81	0.41**	0.89**	67	0.81**	1.2	...	...
1950	HRW	45	0.68**	56	−0.09	0.76**	48	0.93**	0.5	0.62**	60
	HRS	50	0.40**	52	0.20	0.48**	50	0.55**	0.6	0.37**	54
	SRW	267	0.31**	59	0.06	0.40**	57	0.68**	0.5	...	...
	White	100	0.12	51	−0.16	0.36**	48	0.65**	0.5	...	...
	Comb.[d]	462	0.57**	60	0.04	0.64**	56	0.87**	0.5	...	...
1951	HRW	29	0.86**	31	0.39**	0.89**	28	0.88**	0.5	0.48**	54
	HRS	26	0.89**	34	0.23	0.93**	27	0.92**	0.5	0.71**	52
	SRW	13	0.84**	20	−0.03	0.94**	13	0.89**	0.2	...	...
	White	11	0.96**	17	0.42	0.95**	19	0.98**	0.2	...	...
	Comb.[d]	79	0.97**	34	0.49**	0.97**	32	0.97**	0.5	...	...
1952	HRW	106	0.78**	46	0.60**	0.62**	58	0.75**	1.1	0.35**	69
	HRS	60	0.88**	36	0.29**	0.92**	28	0.90**	0.6	0.72**	51
	SRW	78	0.68**	18	0.50**	0.53**	21	0.76**	0.4	...	...
	White	41	0.78**	45	0.09	0.86**	36	0.88**	0.7	...	...
	Comb.[d]	285	0.90**	48	0.56**	0.88**	53	0.89**	0.9	...	...
1953	HRW	126	0.75**	40	0.61**	0.56**	50	0.73**	1.5	0.46**	54
	HRS	77	0.80**	56	0.08	0.93**	34	0.84**	1.0	0.62**	73
	SRW	71	0.16	37	−0.33	0.45**	35	0.78**	0.4	...	...
	White	39	0.60**	45	−0.15	0.79**	36	0.86**	0.6	...	...
	Comb.[d]	313	0.83**	54	0.50**	0.78**	60	0.85**	1.2	...	...
1954	HRW	88	0.82**	41	0.14	0.88**	33	0.89**	0.7	0.60**	57
	HRS	67	0.80**	60	0.23*	0.92**	39	0.81**	1.1	0.70**	70
	SRW	51	0.48**	36	0.28*	0.44**	36	0.66**	0.8	...	...
	White	93	0.70**	40	0.31**	0.76**	36	0.73**	0.7	...	...
	Comb.[d]	299	0.91**	46	0.39**	0.94**	40	0.92**	0.9	...	...
1955	HRW	73	0.83**	41	0.39**	0.85**	38	0.84**	0.9	0.65**	55
	HRS	67	0.65**	48	0.27*	0.80**	38	0.66**	0.9	0.29*	61
	SRW	32	0.89**	27	0.51**	0.86**	30	0.92**	0.7	...	...
	White	38	0.70**	36	0.23	0.76**	33	0.81**	0.8	...	...
	Comb.[d]	210	0.90**	47	0.09	0.93**	39	0.93**	0.8	...	...

[a] Predominant class; see below for distribution.

Group Designation		Distribution, Classes			
Class	Number	HRW	HRS	SRW	White
1947					
HRW	535	485	50	...	...
HRS	560	103	457	...	...
HRW	511	380	50	...	81
HRS	700	211	437	28	24
HRW	79	27	17	23	12
1948					
HRS	677	129	541	...	7

[b] Milling and baking done by three commercial laboratories under contract — each group tested by one laboratory.

[c] Mostly variety samples grown in a plant-breeding program.

[d] Preceding groups of the same year combined.

view is supported by the relatively high coefficients of partial correlation ($r_{sv.p}$) shown for these two groups. It has previously been shown (4) that certain varieties, such as Redchief and Chiefkan, that have inherent inferior gluten quality, nearly always have lower sedimentation values than wheat of normal or superior gluten quality.

Data in Table I also show that the sedimentation value is more reliable than the percentage of dark, hard, and vitreous kernels as an index of baking strength.

Nearly all of the correlation coefficients in Table I are relatively high; however, low coefficients do not necessarily indicate poor correlation. This possibility is verified by the relatively small corresponding standard errors of estimate, which indicate the variations of the y values from the regression.

TABLE II

COMPARISON OF RATINGS OF 534 WHEAT SAMPLES OF THE 1947 CROP BY FARINOGRAPH AND SEDIMENTATION TESTS

	Rated by Farinograph							
	Excellent	Very Good	Good	Fair–Good	Fair	Poor–Fair	Poor	Very Poor
Av. loaf volume	909	859	825	795	799	803	801	758
No. of samples	90	97	84	45	75	70	60	13
	Rated by Sedimentation Values							
	55.0 and Over	50.0–54.9	45.0–49.9	40.0–44.9	35.0–39.9	30.0–34.9	25.0–29.9	Under 25.0
Av. loaf volume	938	910	882	848	812	787	769	688
No. of samples	76	27	49	66	101	129	79	7

TABLE III

CORRELATIONS OF FARINOGRAPH VALORIMETER VALUES AND SEDIMENTATION VALUES WITH BREAD LOAF VOLUMES
(829 Samples of the 1948 crop)

	Class and Number							
	Hard Red Winter 513		Hard Red Spring 265		White 51		All 829	
	r_{xy}	$S_{y.x}$	r_{xy}	$S_{y.x}$	r_{xy}	$S_{y.x}$	r_{xy}	$S_{y.x}$
Valorimeter value (x) vs. loaf volume (y)	0.40	68.0	0.60	57.0	0.56	43.0	0.62	71.0
Sedimentation value (x) vs. loaf volume (y)	0.68	55.0	0.67	53.0	0.80	32.0	0.79	56.0

From the data in Tables II and III it is apparent that the sedimentation test is somewhat superior to the farinograph test as a measure of baking strength as shown by bread loaf volume. Specific quality

factors, such as mixing time, mixing tolerance, and water absorption, obviously are not measured by the sedimentation test as they are by the farinograph test.

If the details of the method as now outlined are carefully followed, it is usually possible to get good interlaboratory agreement. In Table IV are shown the results obtained by nine collaborators working with six samples of wheat. Each result shown is the average of two determinations, most of which agreed within one unit. Eight field offices of the Grain Division took part in this collaboration. The ninth collaborator was one of the authors. Personal instruction was given to the field men before these tests were made, as only two of them were chemists.

TABLE IV

SEDIMENTATION VALUES OBTAINED BY NINE COLLABORATORS ON SIX SAMPLES OF WHEAT

	Sample No.					
Collaborator	526	527	528	529	530	531
1	42	28	67	45	50	10
2	43	26	67	51	50	6
3	43	27	68	51	51	8
4	43	29	67	55	51	5
5	44	28	68	52	51	8
6	46	28	68	51	53	7
7	44	28	68	53	53	9
8	40	28	68	57	55	10
9	46	29	68	57	53	8
Average	43.4	27.9	67.7	52.4	51.9	7.9

Because of the simplicity of the sedimentation test and the speed with which it can be made in comparison with other tests of comparable usefulness, it appears that the test may well be adapted to routine wheat inspection. With this in view, and on the basis of data obtained in studies of large numbers of wheat samples over the past 9 years, the following possible classification of wheat according to sedimentation values is suggested:

1. *Sedimentation Values of 60 and Over.* This wheat consists almost entirely of hard wheat (other than durum wheat) and is nearly always of high protein content, usually over 14%, and of superior gluten quality. It has superior baking strength and is suitable for mixing with weaker wheat for the production of bread flour, or for milling the very strong flour demanded by a small segment of the bread-baking industry. In normal years this type of wheat is in short supply and commands a considerable premium. Wheat of the character of the varieties Redchief and Chiefkan rarely, if ever, falls into this range.

2. *Sedimentation Values of 40 to 59.* This wheat consists almost entirely of hard wheat (other than durum wheat) and is of the type most widely used for production of bread flour. The protein content of the wheat is usually from 12 to 14%, and the quality of the gluten is usually good. Redchief and Chiefkan wheat fall into this range only when of very high protein content and consequently of at least reasonably high bread-baking strength.

3. *Sedimentation Values of 20 to 39.* This wheat consists largely of low protein content hard wheat such as that produced east of the Mississippi River and on the Pacific Coast. Redchief and Chiefkan wheat and other varieties of similar character, even though of fairly high protein content, as well as some of the highest protein content soft wheat also fall in this range. Included also in this range is hard wheat that has suffered gluten injury as a result of unfavorable weather conditions prior to harvest. The wheat is best suited to the production of "all-purpose" flour or for use in mixing with stronger wheat for the production of bread flour.

4. *Sedimentation Values of Less Than 20.* This wheat consists almost entirely of soft wheat but may contain some hard wheat of exceptionally low protein content or exceptionally weak gluten quality. The wheat is used primarily for the production of cake, pastry, cookie, and cracker flours.

Acknowledgment

The authors wish to acknowledge the assistance of the members of the technical staff who have aided in this study.

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APPLICATION OF THE KARL FISCHER METHOD TO GRAIN MOISTURE DETERMINATION[1]

JOE R. HART AND M. H. NEUSTADT[2]

ABSTRACT

A method has been developed for determining moisture in grain which involves the simultaneous grinding of the grain and extraction of the water with methanol, and subsequent titration of the extract with Karl Fischer reagent. Results were in good agreement with those obtained with official oven methods for wheat, oats, barley, rice, and rye, but were somewhat lower than oven method results for soybeans and flaxseed and somewhat higher for pea beans and corn. Volatile substances other than water are removed in the heating of soybeans and flaxseed which give higher moisture values by the oven method, and in the case of corn and pea beans not all of the water is removed by the water-oven method. Essentially all of the water is extracted from the grain by the new method and the extracts contain no appreciable amount of material other than water which will react with the Karl Fischer reagent.

Under the Official Grain Standards of the United States an air-oven method is prescribed for determining the moisture content of wheat, barley, oats, rye, rice, flaxseed, and soybeans; and a water-oven method for corn (11).

Oven methods for moisture determination have been criticized because heating may (a) produce chemical decomposition of organic compounds with the formation of water not originally present, (b) drive off volatile substance other than water, (c) seal in moisture by hardening the surfaces, and (d) cause oxidation which would result in increased weight and thus apparent low moisture values (1). This paper will show that some of these criticisms are valid with respect to certain grains.

Moisture is ordinarily considered as being held in organic materials much as a sponge holds water. However, some of the water is held more firmly because of the forces related to surface phenomena. In addition to water in liquid form there is water of crystallization, water of constitution, and possibly water held by other forces. Some of this water is "free" and some is "bound" according to the definitions sometimes favored for these terms. Regardless of what fraction of the total water we consider to be "free" and what fraction "bound," it is known that the relationship between the water and the dry matter in grain is not

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[2] Grain Division, Agricultural Marketing Service, United States Department of Agriculture, Washington, D. C.

a static one. There is dynamic equilibrium between the water and the material holding it (3). The authors believe that if all of the "free" water in a sample of grain could be removed instantaneously, immediately the system would move toward a new equilibrium in which some water which was formerly "bound" would now be "free." Any attempt to remove total water would result in the upsetting of the equilibria between compounds which lose water on heating and their decomposition products and this would cause removal of water which could not be considered as true moisture. Hence, in methods of moisture determination involving the removal of water from grain, the results obtained will not correspond exactly to the total "free" water, total "free" water plus certain types of "bound" water, or total water, since all of these quantities are changing continuously during the process of water removal. Sair and Fetzer (9) bear out this point in their study of methods of determining moisture in corn, using their reversibility method.

It would appear, then, that the method which would most correctly measure the moisture in grain would be that method in which water removal takes place in the minimum time, at the minimum temperature, and under conditions such that at the end of the removal an equilibrium can be shown to exist between the water vapor pressure of the grain and that of the medium surrounding it when the pressure is at a very low value.

Titration with Karl Fischer reagent has been applied to a wide variety of products including many foodstuffs (5, 10). Dehydrated foods (6), starch (2), flours (2), and other cereal products have been tested. There were no extraction problems involved in these cases since the substances were either already in a finely divided state or were soft and porous so that grinding was easy. The method of grinding-extracting grains described in this paper appears to be superior to those described by Morell and by Mitchell and Smith (7) in that there is very little danger of moisture loss during the process. The titrations were carried out with the samples in contact with the water solvent in the titration flask.

The method proposed here involves extraction of water from the grain with a water solvent and subsequent titration of the extract with Karl Fischer reagent. Time of extraction is short (5 minutes), temperature is low (64.5°C.), and it can be demonstrated that at the end of the extraction the partial vapor pressure of water in the solvent equals the water vapor pressure of the grain. The evidence for this lies in the

fact that further treatment of the grain results in no increase in the amount of water in the solvent.

The advantages of the proposed method lie in the fact that the reagent is specific for the determination of water (except in solutions containing certain classes of compounds whose absence in grain extracts can be demonstrated) and in the fact that in a chemical method the prolonged heating and high temperature of the oven method are avoided. The technique for applying the Karl Fischer method to determination of moisture in wheat, corn, rye, barley, oats, flaxseed, soybeans, pea beans, and rice is described in this paper.

Development of the Method

There were several problems to be solved in applying the Karl Fischer method to grain. Among these were: obtaining completeness of extraction and establishment of a method of verifying it, determining the best solvent and establishing the optimum ratio of solvent to grain, and testing for interfering substances.

It was found by experiment that if different weights of a sample of grain containing approximately 10% of water are each extracted to completion (that is, to equilibrium with the solvent) with the same amount of solvent (200 ml.), and aliquots are taken for titration in each extraction which represent 1 g. of grain, the results show the same moisture content throughout the range from 0 to 35 g. of sample. From Fig. 1, a graphic representation of these results, we observe that if 10 g. is chosen as the size of sample to be used with 200 ml. of

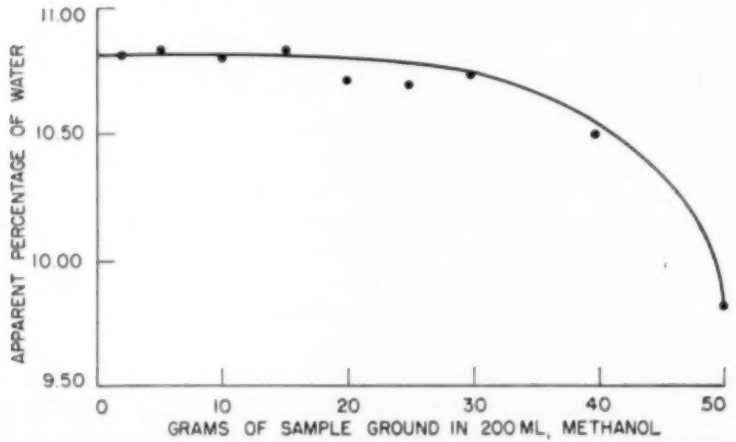

Fig. 1. Relationship between size of sample and completeness of extraction.

solvent in a determination, the maximum extraction is obtained with samples yielding up to 35% of water.

When the extraction procedure is viewed in the light of the law of mass action, it is seen that the results obtained are those to be expected. Although the partial vapor pressure of water in the extract of a sample containing 35% moisture is appreciable, the grain adsorbs both water and solvent on active centers; but water is displaced because of the very much larger amount of solvent present.

The methods of extraction described by Fosnot and Haman (2) and by McComb and McCready (6) are time-consuming and involve the risk of loss of moisture during the grinding process. With high moisture samples a preliminary partial drying would be necessary before grinding, involving additional weighings with increased chances for error. In the method of Fosnot and Haman the samples remain in contact with excess of Karl Fischer reagent for periods of 60 minutes or longer at somewhat elevated temperatures. There is no way of knowing to what extent the reagent deteriorates during this period. A method in which the grain is entirely enclosed and in contact with the water solvent only during the grinding process would be more rapid and would eliminate the danger of moisture loss.

A Stein mill[3] (4) was satisfactory for simultaneously grinding and extracting the grain samples. Considerable heat is developed during the grinding-extracting process. Experience gained while extracting ground grain showed that application of heat hastened extraction of the water. To prevent evaporation of the heated solvent (methanol), a water-jacketed condenser tube was attached through the plate (Fig. 2) which serves as a covering for the cup. A drying tube was connected to the open end of the condenser.

In the grinding process sufficient heat is generated to make the brass parts of the mill and the copper condenser act as catalysts for the oxidation of some of the methanol with the production of formaldehyde and water. It is, therefore, necessary to run a blank titration on the solvent after it has been used in the mill for the same length of time as the grinding of the sample.

Choice of Solvent. In choosing an extraction solvent, methyl "Cellosolve" (methoxy ethanol), ethylene glycol, dioxane, and methanol were investigated. Both dioxane and methyl "Cellosolve" gave moisture values which were distinctly lower than those obtained with methanol. (Dioxane gave values approximately 2% lower and methyl Cellosolve gave values 1.2% lower.) Ethylene gylcol was rejected because of its

[3] The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

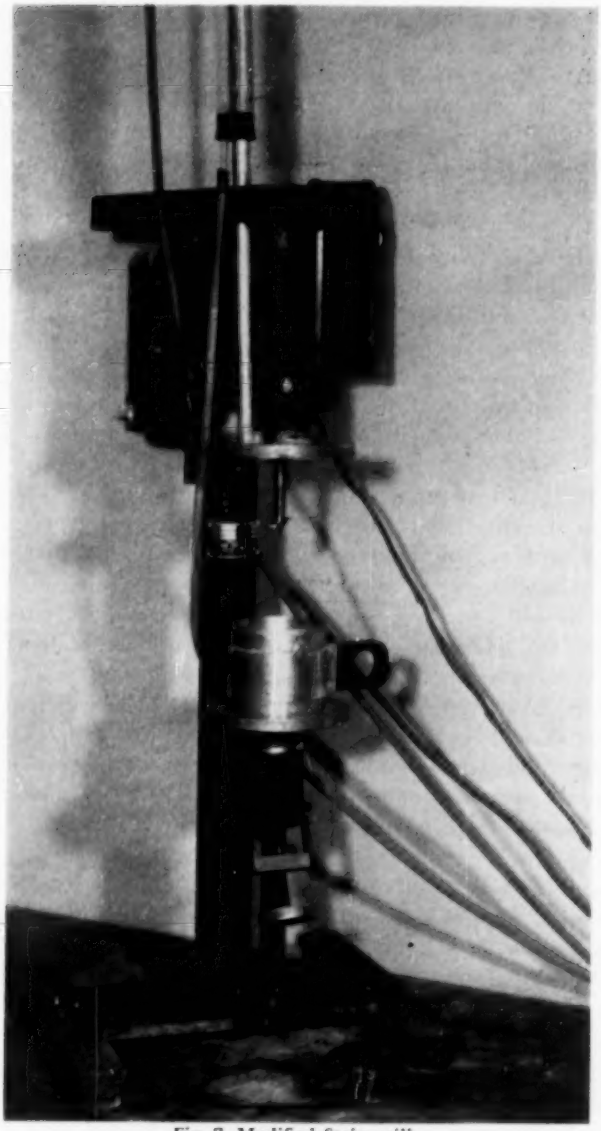

Fig. 2. Modified Stein mill.

high viscosity which makes accurate pipetting difficult and because it forms a colloidal dispersion with ground grain which is very difficult to filter.

The characteristics of the mill used were such that a good wet grind could not be obtained with a liquid having the specific gravity of

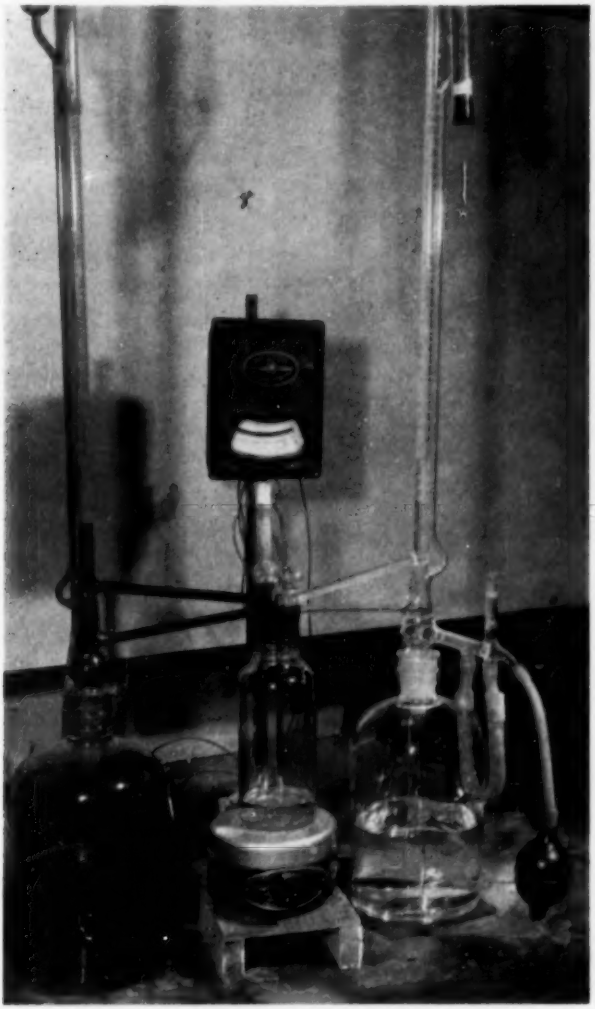

Fig. 3. Titration apparatus for moisture determination.

methanol. Consequently, a preliminary dry grind was necessary for all the grains.

Method. The procedure for making a determination is as follows: Ten grams of grain, weighed with an accuracy of ± 0.005 g., are

TABLE I

SUITABILITY OF THE KARL FISCHER METHOD FOR DETERMINING ADDED WATER

KIND OF GRAIN	SAMPLE NO.	MOISTURE BY KARL FISCHER METHOD	50 G. WETTED 10	MOISTURE IN WETTED GRAIN		DIFFERENCE	AVERAGE DEVIATION
				Calculated	Found		
		%	g.	%	%	%	%
Flaxseed	1	7.0	53.80	13.6	13.6	0.0	
	2	8.8	54.93	17.0	17.0	0.0	
	3	8.8	55.23	17.5	17.6	+0.1	
	4	10.3	55.23	18.8	18.8	0.0	
	5	8.2	55.23	16.9	16.9	0.0	0.0
Soybeans	1	11.4	56.13	21.4	21.4	0.0	
	2	13.6	55.32	21.9	21.8	−0.1	
	3	13.8	55.23	21.9	21.8	−0.1	
	4	9.5	55.25	18.1	18.1	0.0	
	5	13.9	58.49	26.4	26.6	+0.2	
	6	13.8	56.97	24.3	24.1	−0.2	0.0
Pea beans	1	16.1	61.27	31.6	31.7	+0.1	
	2	15.5	53.92	21.6	21.7	+0.1	0.1
Eastern barley	1	15.6	56.89	25.8	25.9	+0.1	
	2	11.2	56.83	21.9	22.1	+0.2	
	3	13.8	56.73	23.9	23.8	−0.1	
	4	12.3	55.98	21.6	21.7	+0.1	
	5	14.2	56.83	24.5	24.5	0.0	0.1
Oats	1	11.7	57.82	24.8	25.0	+0.2	
	2	11.0	56.59	21.4	21.2	−0.2	
	3	11.3	56.91	20.8	20.8	0.0	
	4	11.2	56.14	20.9	20.7	−0.2	0.1
Rye	1	13.7	56.37	23.3	23.2	−0.1	
	2	11.5	56.42	21.5	21.3	−0.2	
	3	12.7	57.09	23.6	23.7	+0.1	
	4	12.0	56.65	22.3	22.5	+0.2	
	5	12.2	56.65	22.5	22.6	+0.1	
	6	12.0	57.12	22.9	22.7	+0.3	0.1
Corn	1	19.6	59.66	32.6	36.7	+0.1	
	2	23.5	60.31	36.6	36.6	0.0	
	3	14.7	57.01	23.2	23.1	−0.1	
	4	17.1	55.13	24.4	24.3	−0.1	
	5	15.9	55.35	23.5	23.7	+0.2	
	6	16.7	54.82	24.0	24.1	+0.1	
	7	22.0	59.70	34.2	34.1	−0.1	
	8	19.6	59.62	32.5	32.6	+0.1	0.0
Hard red winter wheat	1	11.1	55.67	20.2	20.2	0.0	
	2	11.9	57.07	22.8	22.8	0.0	
	3	12.8	55.82	21.9	22.1	+0.2	
	4	12.5	55.87	21.7	21.6	−0.1	0.0
Soft red winter wheat	1	11.3	56.03	20.8	20.9	+0.1	
	2	11.8	56.53	22.1	22.2	+0.1	
	3	12.8	56.53	22.9	22.8	−0.1	0.0
Hard red spring wheat	1	12.6	55.27	21.0	21.2	+0.2	
	2	14.9	55.44	23.2	23.2	0.0	
	3	12.5	56.69	22.8	22.6	−0.2	
	4	13.3	57.10	23.1	23.1	0.0	
	5	13.2	56.70	23.5	23.5	0.0	
	6	11.0	55.52	20.0	20.1	+0.1	0.0
Western white wheat	1	12.6	56.20	22.3	22.4	+0.1	0.1
Durum wheat	1	12.1	56.39	22.0	22.0	0.0	
	2	13.8	56.34	23.5	23.4	−0.1	
	3	13.5	57.00	24.1	24.1	0.0	
	4	15.3	56.97	25.7	25.8	+0.1	
	5	14.6	56.59	24.6	24.5	−0.1	0.0

ground in the Stein Mill for at least 3 minutes. Two-hundred milliliters of methanol are then added through the condenser tube and grinding is continued for 5 minutes. The cup is cooled after the grinding by placing a pan of cold water underneath it. The cup contents

are decanted into a hydrometer jar and allowed to stand until suspended matter has settled to the bottom (5 to 15 minutes, depending on the material tested). A 20-ml. aliquot is then pipetted into the titration cell, 40 ml. of methanol are added, and the titration is made.

The titration is carried out with reagent prepared according to Peters and Jungnickel (8). The dead-stop back-titration method of Wernimont and Hopkinson is used (12, 13). The standard water-in-methanol solution is prepared and standardized according to the method described by Mitchell and Smith (7). The simplicity of the titration set-up is shown in Fig. 3.

Completeness of Extraction. To check completeness of extraction and freedom from error in the Karl Fischer determination (7), a moisture determination is first made on a sample. A fixed quantity of water is then added to another weighed portion of the sample. This portion is allowed to stand for a few hours until the water has been taken up and there is no evidence of surface moisture. It is weighed again. On the assumption that the original moisture determination was correct, and from the gain in weight, the moisture content of the wetted portion is calculated. This calculated value should agree with the determined value for the wetted portion. The results of this study are given in Table I.

Testing for Interfering Substances. It was necessary to determine whether there were substances other than water present in the grain extracts which would react with Karl Fischer reagent. A methanol extract of each kind of grain was prepared and placed in a 150-ml. flask.

TABLE II

COMPARISON OF KARL FISCHER METHOD WITH THE OFFICIAL OVEN METHODS FOR GRAIN

GRAIN	NO. OF SAMPLES	OVEN METHOD	K.F. METHOD	AVERAGE DEVIATION	STANDARD DEVIATION
		%	%	%	%
Western white wheat	35	12.08	12.01	+0.07	0.15
Eastern white wheat	19	12.19	12.11	+0.08	0.15
Durum wheat	28	13.53	13.55	−0.02	0.19
Soft red winter wheat	32	12.34	12.27	+0.07	0.16
Hard red winter wheat	32	10.88	10.88	0.00	0.03
Hard red spring wheat	27	12.44	12.47	−0.03	0.24
Rice, rough and milled	18	12.45	12.53	−0.08	0.17
Flaxseed[a]	32	7.48	6.86	+0.62	0.71
Oats	33	11.21	11.12	+0.09	0.32
Eastern barley	29	13.56	13.43	+0.13	0.30
Rye	43	12.55	12.54	+0.01	0.18
Soybeans	31	9.85	9.29	+0.56	0.57
Corn	46	16.21	17.35	−1.14	1.20
Pea beans	44	15.56	16.47	−0.90	1.03

[a] Whole seed; 3 hours; 130°C.

The extract was evaporated to dryness on a steam bath in a stream of nitrogen gas and then held in a desiccator containing phosphorus pentoxide and a nitrogen atmosphere until constant weight was obtained. Fifty milliliters of methanol were added and the solution titrated with Karl Fischer reagent. The titration results for the extracted material were equivalent to those obtained for the blank determination. The absence of aldehydes and ketones was shown by appropriate chemical tests. Other volatile interfering substances such as amines and amino alcohols were presumed to be absent.

Results

The moisture values obtained for each grain are given in Table II. A comparison is made between the average moisture content obtained by the Karl Fischer method with that obtained on the same samples by the official oven methods. Flaxseed (whole) heated in the convection oven at 130°C. for 3 hours was the standard method for its comparison. The average deviation and the standard deviation of the oven method from the Karl Fischer method for each grain are given.

There is good agreement between the two methods except in the cases of flaxseed, soybeans, corn, and pea beans. Experiments were run to find the reason for the higher results obtained with the oven method on flaxseed and soybeans. A ground sample of each was placed in a flask, the flask was placed in a convection oven, and connections were made to the flask through a hole in the top of the oven with glass inlet and outlet tubes. A slow stream of carbon dioxide-free nitrogen was passed through the flask as it was heated at 130°C. The outlet was connected to a gas wash bottle which contained freshly prepared clear barium hydroxide solution. Precipitates of barium carbonate were obtained with these grains, whereas no precipitates were obtained when other grains were similarly tested. With soybeans and flaxseed an oily liquid condensed in the tube outside the oven which gave a positive test for phosphate and was presumed to be phospholipids. In addition, when flaxseed was run, a dark ring of charred substances appeared in the tube at the point where it emerged from the oven.

The explanation for the discrepancy between the oven results and those obtained with the Karl Fischer method for corn and beans was derived from other experimental work. As was mentioned previously, the official method for these two commodities is the water-oven method. Although the temperature of the water oven is 100°C., samples heated in the water oven and in an air-convection oven at the same temperature do not yield the same results. In fact, corn samples at constant weight in the water oven will lose weight when placed in an air oven

at 100°C.; whereas if the procedure is reversed, the samples will gain weight. The actual difference between the air-oven method at 103°C. for 96 hours, the water-oven method, and the Karl Fischer method for corn and beans is shown in Table III. The average difference between the results at 100°C. and 103°C. in the air oven is 0.3%.

TABLE III

COMPARISON OF AIR-OVEN AND WATER-OVEN METHODS WITH THE KARL FISCHER METHOD FOR CORN AND PEA BEANS

GRAIN	NO. OF SAMPLES	AVERAGE MOISTURE CONTENT			AVERAGE DEVIATION		
		103° Air-Oven Method (1)	Water-Oven Method (2)	Karl Fischer Method (3)	(1)–(2)	(1)–(3)	(2)–(3)
		%	%	%	%	%	%
Corn	65	17.36	15.88	17.14	+1.44	+0.22	−1.26
Pea beans	27	12.68	11.49	12.61	+1.19	+0.07	−1.12

The discrepancy between the water-oven results and those obtained by the Karl Fischer method for corn and beans was due to the fact that heating such samples in the water oven for 96 to 120 hours does not remove all of the water. Moisture determinations were made by the Karl Fischer method on samples of corn and beans which had been dried in the water oven by the official method. The results indicated that the amounts of water still present were actually greater than the differences between the Karl Fischer method results and the water-oven method results. Appropriate chemical tests indicated the presence of amines in samples coming from the water oven, even though they were absent in the unheated samples. To avoid interference from these amines the Karl Fischer titrations of the heated samples were carried out in the "spent reagent" from previous titrations according to the method of Mitchell and Smith (7), in order to determine only the

TABLE IV

COMPARISON OF KARL FISCHER AND WATER-OVEN METHODS FOR MOISTURE IN CORN AND BEANS

KIND	KARL FISCHER METHOD	WATER-OVEN METHOD	DIFFERENCE	KARL FISCHER METHOD ON SAMPLES AFTER WATER-OVEN DRYING
	%	%	%	%
Corn	16.16	14.85	+1.31	1.99
Corn	11.89	10.85	+1.04	1.88
Corn	14.21	13.11	+1.10	1.81
Red kidney beans	8.21	7.98	+0.22	1.11
Pea beans	11.44	10.61	+0.83	1.14

water which was present. Thus, it is shown that, although the water-oven method gives low results compared to the Karl Fischer values, the amount of water indicated as being present is actually in excess of the amount removed from the sample by heating. The results of these tests are given in Table IV.

Discussion

These studies show that the modified Karl Fischer method for moisture determination may be considered a basic method for moisture determination in grain. The method is capable of determining essentially all of the water in the grain and there are no significant amounts of substances in the grain which will react with the reagent to produce erroneous moisture results. The previous choice of official air-oven methods for grains was fortunately a judicious one, since the agreement in most instances is very good between the Karl Fischer method and the official method. In those instances where the agreement is not so good, the official oven method was in error as it gave rise to volatile materials other than water. The official water-oven method for moisture content of corn and beans does not remove all of the moisture.

Acknowledgment

The writers are indebted to W. Haward Hunt who performed the oven-moisture tests described in this paper.

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INTERACTIONS BETWEEN PROTEINS AND POLYSACCHARIDES OF WHEAT FLOUR[1]

Doyle C. Udy[2]

ABSTRACT

The flow properties of dispersions of wheat flour in dilute acid are influenced primarily by an interaction between the dispersed polysaccharides and gluten proteins. This interaction depends to a large extent upon the quality of the polysaccharides present, but also upon the quantity and quality of the gluten proteins.

Gels will form under suitable conditions, and they exhibit both syneresis and thixotropy.

The interaction is not observed when a solution of 8% sodium salicylate is used as the solvent. Minute quantities of thioglycolic acid or sodium bisulfite also prevent gel formation. In contrast, oxidizing agents very markedly promote the interaction.

Heating of the polysaccharides in dilute acetic acid caused a sharp decrease in their tendency to interact with gluten. Dispersions of gluten gave a similar, but less pronounced, response to heat treatment.

Soluble polysaccharides of high molecular weight, which have intrinsic viscosities of about 3.8 deciliters/g. or greater, display appreciable interaction with gluten. Polysaccharides of low molecular weight show very little interaction.

In an earlier investigation (10) it was noted that the soluble polysaccharides accounted for nearly two-thirds of the intrinsic viscosity of dilute acid extracts of flour even though they represented only 1% of the sample weight. Since wheat proteins give much lower intrinsic viscosities than do the soluble polysaccharides, an increase in the ratio of proteins to polysaccharides should *decrease* the observed intrinsic viscosity. Yet, it is commonly observed, and Bresson and Barmore (3) have recently recorded extensive data showing, that viscosities of dilute acid dispersions of flour increase much more than would be expected

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[2] Chemist, Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture, Pullman, Wash.

with small increases in the protein content of samples within a given variety of wheat. It is not uncommon to find correlation coefficients as high as 0.9.

In many samples, the viscosities of dilute acid dispersions of flour, as measured by the procedure described in *Cereal Laboratory Methods* (1), are about ten times greater than would be expected theoretically. A partial or complete explanation of these apparent anomalies lies in an interaction between the gluten proteins and the soluble polysaccharides of wheat. This paper considers some of the factors involved in this interaction by means of intrinsic viscosity changes occurring in mixtures of polysaccharides and glutens when subjected to various environments and experimental conditions while in solution.

Materials and Methods

Straight-grade, unbleached flour from three pure wheat varieties, Rio, Baart, and Elgin, was used for the viscosity measurements of flour dispersions and as a source of the polysaccharides and glutens for other viscosity measurements. They represent strong, medium, and weak types of flour, respectively, and the wheats were all grown at Pullman, Washington, in 1954. Other data describing these flours on a 14.0% moisture basis are tabulated below:

Variety	*Type*	*Protein*	*Ash*
		%	%
Rio	Hard red winter	10.8	0.30
Baart	Soft white	9.8	0.36
Elgin	Club	9.0	0.34

Preparation of Soluble Polysaccharides. A quantity of flour was mixed with four parts of distilled water and then agitated in a Waring Blendor for 4 minutes to effect solution of the soluble components. After 10 minutes' centrifuging, the extract was treated by three separate procedures. In the first, the extract was made 0.05*M* with respect to acetic acid. This solution was then passed through a coil of 4-mm. glass tubing immersed in boiling water to inactivate enzymes that would otherwise degrade the polysaccharides during viscosity measurements. The solution was retained within the glass coil for about 20 seconds. Acidification prevented the proteins from flocculating during the heat treatment. The heat-treated extract was immediately cooled, and 6 g. of Special Filtrol[3] were added for each 100 ml. of solution. After 30 minutes' shaking, the mixture was centrifuged, and two volumes of acetone were added for each volume of extract. Approxi-

[3] Special Filtrol is one of several activated clays marketed by the Filtrol Corporation, Los Angeles, California.

mately 85% of the soluble proteins (N × 5.7) was adsorbed by the Filtrol, and about half of the remaining nitrogen was precipitated along with the polysaccharide fraction. The latter was dried in air. In another preparation the acidification and heating steps were omitted; otherwise, the procedure was the same.

In a third preparation, a barium hydroxide-zinc sulfate reagent was used to selectively precipitate the proteins according to the method used by Pence *et al.* (6).

Polysaccharides prepared by the first two methods contained about 8% soluble protein. Intrinsic viscosities of their aqueous solutions differed only between varieties. These values were 4.2, 3.1, and 2.8 deciliters/g., respectively, for the Rio, Baart, and Elgin flours. A preparation by the barium-zinc method from Rio flour contained 1.5% protein and had an intrinsic viscosity of only 2.3. These values are in accord with molecular weight determinations for similar products prepared by Pence *et al.* (6). Analytical studies on similar preparations have been reported in some detail by Perlin (8) and Gilles and Smith (5).

Fractionation of Flour. These same flours were separated into starch, gluten, and water-soluble fractions by a simplified technique as follows: after the soluble extract was recovered, as described above, the gluten layer was removed from the centrifuge bottle, placed in a dough mixer, and treated with successive increments of distilled water until free of starch. The starch that was washed out with each addition of water was centrifuged and recombined with the starch obtained in the first step. This total starch fraction was then rewashed, centrifuged, and dried in air. The crude gluten was also dried in air and then ground in a Hobart mill at the finest setting. The soluble fraction was recovered by lyophilization.

Measurement of Viscosity. The procedure described in *Cereal Laboratory Methods* (1) was used to measure viscosities of both normal and reconstituted flour dispersed in water and lactic acid. After the initial viscosity measurement had been made, these flour dispersions were agitated in a Waring Blendor for 2 minutes. The dispersions were then allowed to stand for two 30-minute periods. Viscosity measurements were again made at the end of each rest period.

A Ubelohde-type capillary viscometer was employed to determine the ratio of the flow time of polysaccharide, or polysaccharide-gluten, solutions to the flow time of the solvent. This ratio will be referred to as the relative viscosity, η_r, of the solution. Intrinsic viscosity $[\eta]$, is defined by the relationship, $[\eta] = \lim_{C \to O} (\ln \eta_r)/C$, where C is the concentration of total solids expressed in g/100 ml. of solution. For the solu-

tions studied here, $(\ln \eta_r)/C$ was constant, which considerably simplified the determination.

Results and Discussion

Preliminary observations indicated that the viscosities of dispersions of some flours in dilute acid were from 6 to 12 times as much as expected. Although the contribution of all soluble components to the total viscosity could be calculated, large deviations existed which could not be accounted for. Furthermore, the extent of these deviations was characteristic of the variety of wheat from which the flour was obtained.

Interchange of Fractions of Reconstituted Flour. One means of identifying the constituents responsible for the enhanced resistance to flow is to reconstitute and interchange the principal flour fractions. Table I shows the results obtained from such tests.

TABLE I

VISCOSITIES OF DILUTE ACID DISPERSIONS OF NORMAL AND RECONSTITUTED FLOURS BEFORE AND AFTER TREATMENT IN A WARING BLENDOR, AND THE EFFECT OF INTERCHANGE OF FLOUR FRACTIONS

FLOUR SAMPLE	NORMAL[a]	AFTER BREAKDOWN[b]	AFTER 30 MINUTES' REST	AFTER 1 HOUR'S REST
	°*MacM.*	°*MacM.*	°*MacM.*	°*MacM.*
Elgin (normal)	48	8	8	8
Baart (normal)	104	9	8	8
Rio (normal)	210	17	36	70
Elgin (reconstituted)	18	9	10	11
Baart (reconstituted)	28	11	10	10
Rio (reconstituted)	46	27	150	300
Elgin S and G + Rio Sol[c]	32	19	130	...
Rio S and G + Elgin Sol	23	14	14	14
Elgin S and Sol + Rio G	24	10	10	11
Rio S and Sol + Elgin G	37	21	140	...
Baart G and Sol + Rio S	30	13	11	10
Baart S and Sol + Rio G	23	10	11	11
Baart S and G + Rio Sol	35	20	135	...

[a] Viscosity values of flour determined by the method cited.
[b] The unstable structural viscosity was broken down by treatment in a Waring Blendor for 2 minutes.
[c] S, G, and Sol are abbreviations for starch, gluten, and soluble fractions, respectively.

It is evident that the reconstitution was not absolute. Nevertheless, the reconstituted values retain their relative order, and an interchange of fractions gave results which clearly show that the solubles fraction from Rio flour was essential for reforming a high viscosity once the initial unstable structural viscosity had been broken down in the Waring Blendor. Dispersions having viscosities exceeding 100 MacMichael units had visible stringy, gellike structures. Where this struc-

ture appeared, it could be broken down and re-formed in a reversible manner; i.e., it was thixotropic.

Viscosities of Gluten and Polysaccharide Mixtures. An earlier study (10) showed that soluble polysaccharides usually were more abundant in Rio and other hard red winter wheats than in softer varieties, and that the polysaccharides from hard varieties had much larger intrinsic viscosities; i.e., their molecular-weight average was greater. On the other hand, the soluble proteins, which represent the other chief component of the solubles fraction, had nearly the same molecular size in all varieties. In the light of this information, it appeared most likely that the polysaccharide portion of the solubles fraction was involved in the differences observed.

Where interaction between components is not involved, the intrinsic viscosity of mixtures of substances having high molecular weight can be described by the following equation:

$$[\eta] = \sum_{i=1}^{n} [\eta]_i W_i \qquad (1)$$

where $[\eta]_i$ is the intrinsic viscosity of component i and W_i is its weight fraction in the mixture. This relationship has been shown to be valid for mixtures of polysaccharides and proteins (10). Conversely, when there are interactions between components, the measured and calculated values of $[\eta]$ will differ.

Viscosity measurements were made on several mixtures of polysaccharides and glutens prepared from wheat flours having a wide range in viscosity behavior. Values for each mixture were also calculated by the above method. The results of these measurements and calculations are given in Table II.

The first five lines of Table II give values for the single components, whereas the remainder are mixtures. Lines 6 through 11 show large differences between measured and calculated values, while the measured values recorded in lines 12 through 15 are not much greater than those calculated. Differences in these values are significant only within a single mixture or where the ratio of crude gluten to polysaccharides remains unaltered. The calculated value serves as the reference point. The data presented in this table may be interpreted as an indication of an interaction between polysaccharides and gluten. The extent of this interaction depends upon the molecular size of the polysaccharides and also some factor, possibly an enzyme, that is sensitive to heat. This factor plays some part in the mechanism of the interaction. Pence *et al.* (6) found that the soluble proteins (a likely enzyme source) were very influential in the baking performance of reconstituted doughs.

TABLE II

INTRINSIC VISCOSITY VALUES FOR COMBINATIONS OF SEVERAL POLYSACCHARIDE AND CRUDE GLUTEN PREPARATIONS

DESCRIPTION OF MIXTURE	CONCENTRATION	POLYSACCHARIDES	GLUTEN PROTEIN	INTRINSIC VISCOSITY				
				Measured				
				Components Unheated	Polysaccharides Heated	Crude Gluten Heated	Components Heated	Calculated
	g/100 ml	*wt. fract.*	*wt. fract.*					
1. Rio polysaccharides	0.500	1.00	...	4.16	4.15	...	...	...
2. Rio polysaccharides	0.503	1.00	...	...	4.31	...	...	...
3. Baart polysaccharides	0.582	1.00	...	3.10	...	...	...	...
4. Elgin polysaccharides	0.528	1.00	...	2.82	...	...	...	...
5. Gluten	5.26	...	1.00	0.40	...	...	...	...
6. 1[b] + Rio gluten	0.307	0.89	0.11	4.50[c]	...	3.90	...	3.74
7. 1 + Rio gluten	0.372	0.67	0.33	3.54[c]	...	3.43	...	2.92
8. 1 + Elgin gluten	0.342	0.74	0.26	4.01	...	...	...	3.18
9. 1 + Baart gluten	0.330	0.79	0.21	5.08[c]	3.42	3.76	3.37	3.36
10. 2 + Rio gluten	0.391	0.86	0.14	...	...	...	3.85	3.75
11. 2 + Rio gluten	0.357	0.70	0.30	...	3.41	...	3.22	3.14
12. 3 + Rio gluten	0.391	0.70	0.30	2.28	...	...	...	2.28
13. 3 + Baart gluten	0.351	0.70	0.30	2.54	...	...	...	2.28
14. 4 + Elgin gluten	0.351	0.75	0.25	2.61	...	...	...	2.25
15. Rio polysaccharides (barium-zinc prep.) + Rio gluten	0.388	0.77	0.23	...	1.91	...	1.91	1.90

[a] The heat-treatment of polysaccharide preparations is described in the Methods section. Crude gluten was similarly heat-treated after dispersion in 0.05*M* acetic acid.

[b] Refers to above mixture number.

[c] Flow through the capillary was slightly irregular owing to partial gel formation.

Change in Viscosity with Time. The measured intrinsic viscosities recorded in Table II were made immediately after the components were mixed together. Those mixtures, which showed a partial gel formation at the start (see A in Fig. 1), continued to increase in viscosity until a fairly continuous gel structure resulted. Gelation did occur in mixtures where either component was heat-treated, but the lapse in time between mixing and gelation was considerable. Heat-treatment of the polysaccharide solution (curve C, Fig. 1) was more effective in prolonging the gelation time than was heat-treatment of the gluten dispersion (curve B, Fig. 1). When both components were heat-treated, the measured and calculated intrinsic viscosities were equal. Furthermore, this value remained constant throughout the course of the experiment (curve D, Fig. 1).

Once the mixture begins to show a substantial change in viscosity, the flow properties are sharply altered, and the gel structure becomes

visible. This is typical of crosslinking between components of high molecular weight leading to gel formation (4). At the present time it is not known what sites are involved in the interaction, or how their activity is influenced by heating. The thixotropic property of the gel

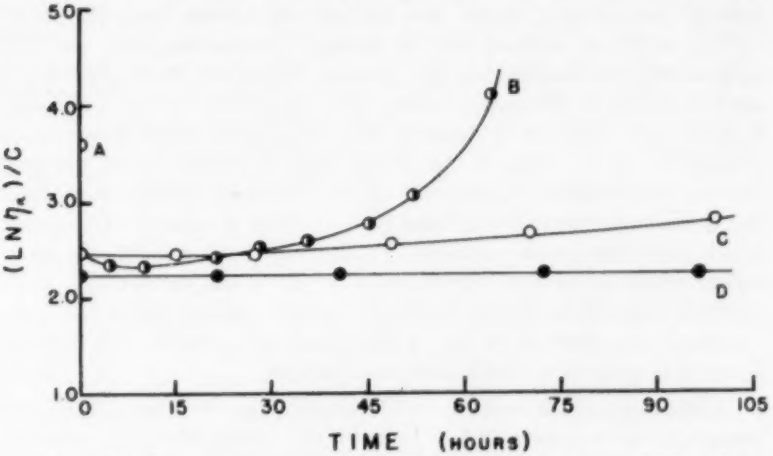

Fig. 1. Effect of heat-treatment on the gelation time of mixtures of Rio polysaccharides (0.5% solution) and Baart gluten (0.5% solution). Point A, neither component heat-treated; curve B, gluten heat-treated; curve C, polysaccharides heat-treated; curve D, both components heat-treated.

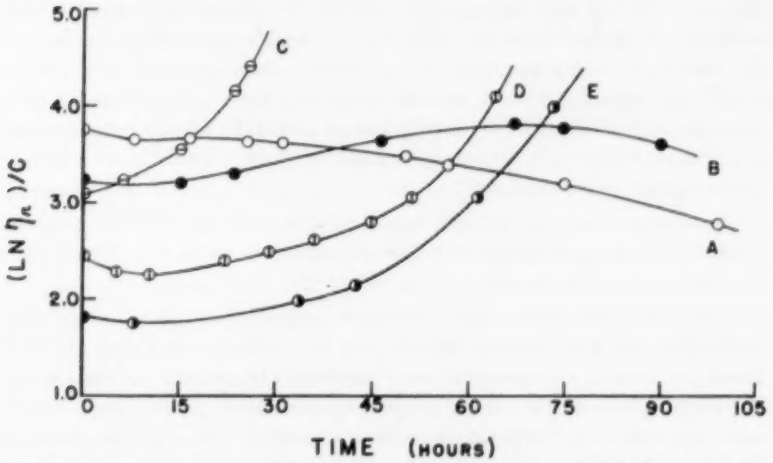

Fig. 2. Effect of gluten concentration on the time required for gelation for mixtures of Baart gluten (heat-treated) and Rio polysaccharides. The weight fraction of gluten was 0.20, 0.30, 0.40, 0.50, and 0.60 for curves A, B, C, D, and E respectively. Solution concentrations ranged between 0.33 and 0.62 g/100 ml.

suggests that the interacting forces are weak and in this respect similar to gelatin.

Another interesting aspect of the interaction is the effect of gluten concentration upon the gelation time. There appears to be an optimum ratio of the components for best interaction. Gluten from the Baart variety of wheat showed slightly stronger interaction with the Rio polysaccharides than did the Rio gluten. Therefore, Baart gluten was used in the series of measurements shown in Fig. 2. Curves A through E show how a gradual increase in the weight fraction of heat-treated gluten (0.2 to 0.6) affects the interaction as measured by viscosity changes. It is evident from the initial decrease and subsequent increase in viscosity that there are at least two opposing reactions taking place which affect the measured viscosity value. On the one hand, the interaction tends to increase the viscosity as the result of building up a network structure of higher molecular weight. On the other hand, an enzymatic degradation of the polysaccharides lowers the viscosity by creating fragments of lower molecular weight.

A thick gel was formed in each reaction mixture when gluten was present to the extent of 0.4 or more of the weight fraction. Syneresis occurred upon further standing for 2 to 3 days.

Effect of Oxidizing Agents. Baker *et al.* (2) obtained gels when oxidizing agents were added to purified gums obtained from the water-soluble extract of wheat flour. Some of the polysaccharide preparations used in the present experiments could be gelled by adding small amounts of iodine. However, the polysaccharide concentrations had to be considerably greater than those used in the mixtures, as found in Table II, before gelation would occur. Furthermore, polysaccharide preparations differed in gel formation (Table III). Of the preparations studied, only Rio polysaccharide solutions (0.5%) would gel when oxidizing agents were added.

Potassium bromate powder was added to several different polysaccharide and gluten mixtures to yield gelatinous products. The results of these experiments are given in Table III.

The addition of bromate greatly accelerated the gelation process, as will be noted by comparing the data for mixtures in Table III with those for similar mixtures (without bromate) illustrated in Figs. 1 and 2. Mixture 2 in Table III gelled in 6 hours and 27 hours, respectively, as compared with 105 hours for curve C and no gel at all for curve D in Fig. 1. Mixture 6 in Table III gelled in 20 minutes, whereas a comparable mixture represented by curve D in Fig. 2 gelled in about 65 hours.

TABLE III

EFFECT OF POTASSIUM BROMATE[a] ON MIXTURES OF POLYSACCHARIDES AND GLUTEN

DESCRIPTION OF MIXTURE	CONC.	GLUTEN	TIME TO GEL			
			Components, Unheated	Polysaccharides, Heated	Gluten, Heated	Components, Heated
	g/100 ml	*wt. fract.*	*hours*	*hours*	*hours*	*hours*
1. Rio PS[b] + Rio G	0.375	0.282	...	6.5	...	...
2. Rio PS[b] + Rio G	0.357	0.300	...	6.0	...	27
3. Rio PS[b] + Rio G	0.398	0.076	...	64.0	...	...
4. Rio PS[b] + Rio G	0.365	0.080	...	...	...	70
5. Rio PS[b] + Rio G	0.340	0.118	0.4	...	0.5	...
6. Rio PS[b] + Rio G	0.372	0.327	immediate	...	0.3	...
7. Rio PS[c] + Rio G	0.388	0.230	...	no gel	...	...
8. Baart PS + Baart G	0.416	0.396	4	...	...	...
9. Elgin PS + Baart G	0.423	0.390	no gel	...	...	...
10. Rio PS	0.510	...	immediate	...	...	...
11. Baart PS	0.502	...	no gel	...	...	...
12. Elgin PS	0.517	...	no gel	...	...	...
13. Rio PS[c]	0.508	...	...	no gel	...	...

[a] Each solution was made 0.005*M* with respect to potassium bromate.
[b] PS and G refer to polysaccharides and gluten, respectively.
[c] Polysaccharides prepared by barium-zinc method.

Effect of Thioglycolic Acid. Gluten dispersed in dilute acetic acid will gel (7, 9) when small quantities of thioglycolic acid, monothioglycol, or sodium bisulfite are added. Thioglycolic acid was added to mixtures of polysaccharides and glutens to test the possibility that reducing groups on the carbohydrates may be instrumental in effecting the observed interaction. If this were the case, the thioglycolic acid should enhance the gelation process. The results of such measurements do not support this view, since an interaction was not observed. All intrinsic viscosity measurements made in the presence of thioglycolic acid (0.005*M*) were identical to those calculated from equation (1), where otherwise the conditions were optimum for rapid gel formation.

Effect of Sodium Salicylate and Urea. Additional information on the nature of the interaction between gluten proteins and polysaccharides was obtained by using an 8% solution of sodium salicylate as the solvent. This system has a high ionic strength and should inhibit interactions arising from electrostatic forces. Mixtures which readily gelled in dilute acetic acid failed to show any enhanced viscosity that would suggest an interaction between the components when dispersed in the aqueous salicylate solvent. Again, the measured intrinsic viscosities were the same as those calculated from equation (1). Urea

(2*M*) caused firm gels from polysaccharides and gluten to liquefy when they were shaken gently.

General Discussion

These results support the view that weak secondary molecular forces are involved in an interaction between gluten proteins and soluble polysaccharides of wheat. All measurements point to the molecular size of the polysaccharides as being the most important single factor in the interaction. The role of oxidizing agents as flour dough improvers may be intimately associated with the mechanism of this interaction. To the extent that this interaction may occur in doughs or batters prepared from different flours used in baking, it could be an important factor in the quality of the product.

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MICROFLORA OF MILLED RICE[1]

HIROSHI KURATA,[2] KAZUO OGASAWARA,[3] AND VERNON L. FRAMPTON[4]

ABSTRACT

Milled rice produced commercially in the Southern area of the United States in 1954 was virtually free from internal infection by fungi, and it was free from internal infection by bacteria, yeasts, and actinomycetes.

The thesis that microorganisms are normally present within the seeds of the cereal grains, of soybeans, and of cottonseed has been advanced and supported by several workers (1, 2, 4, 5, 6, 7, 8). Some of the authors have stated that the fungi *Aspergillus flavus, A. glaucus,* and *A. candidus* are indigenous to normal wheat (7). According to one report (4), 50% of the rice harvested in the Southern Area of the United States is internally infected by *Aspergillus niger, A. glaucus,* and by *Penicillium* species.

A comprehensive study on rice, carried out at the Southern Regional Research Laboratory, indicated that milled rice produced in the southern parts of the United States in 1954 was not normally infected internally by fungi or bacteria; as a matter of fact, very few indeed of the rice kernels used in this study were found to be infected internally. The results recorded in this communication on the microflora of milled rice flowing in commercial channels show that the number of kernels harboring internal infection is insignificant.

Source of Rice

The milled rice used in this study was representative of the 1954 crop (2.6 million tons) produced in the Southern rice-producing area of the United States. The samples were taken from milled rice flowing in commercial channels; they were collected by the Grain Division, Agricultural Marketing Service, U. S. Department of Agriculture, with the techniques normally employed for collecting samples for grading and inspection. The rice was delivered by messenger to the Southern Regional Laboratory as 1-lb. samples sealed in cartons. In most instances the samples were collected within a week after milling, and they were examined immediately.

[1] Manuscript received May 21, 1956.

[2] Division of Pharmacology, Laboratory of Plant Pathology, National Hygienic Laboratory, Ministry of Health and Welfare, Tokyo, Japan.

[3] Food Sanitation Section, Hokkaido Institute of Public Health, Hokkaido, Japan.

[4] Southern Regional Research Laboratory, New Orleans, Louisiana, one of the laboratories of the Southern Utilization Research Branch, Agricultural Research Service, U. S. Department of Agriculture.

The Southern rice-producing region of the United States is divided by the Grain Division into 11 geographical areas (Fig. 1) for the purpose of facilitating the inspection and grading of rice. Six samples were collected from each area in two series of three samples each. In addition, five 1-lb. samples, U. S. Grade No. 5, were secured from the Crowley, Louisiana, area.

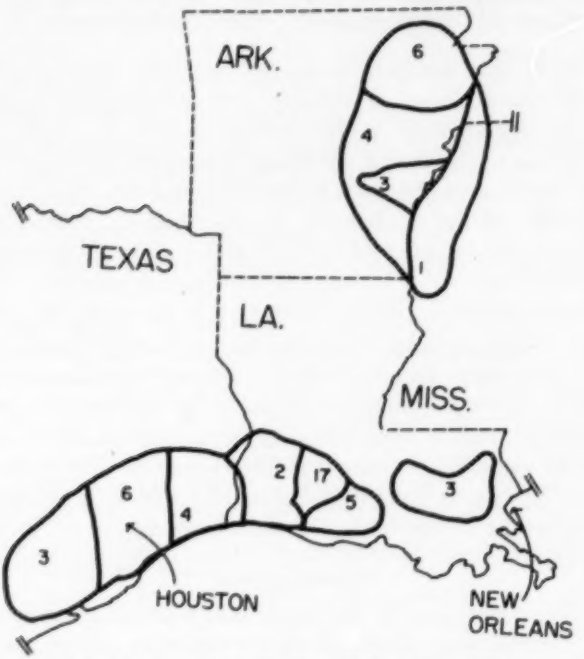

Fig. 1. Rice-producing areas of the Southern United States. The numeral in each indicates the number of rice mills in that area.

Rice grown in the Southern Area is frequently fumigated with methyl bromide. It is not known whether the samples studied had been so fumigated.

Laboratory Sampling

The laboratory operation included a further sampling of the rice specimen supplied. Each 1-lb. lot of the two series was quartered, and a portion from each quarter was spread over cross-section paper containing 100 squares. A kernel was taken from each square, together with a kernel from each square cut by the diagonals. Thus a subsample consisting of 120 kernels of rice was prepared. The three sub-

samples, from a given area, in the first series were combined to form a composite to represent rice from the area in question. A second composite was also prepared from the three subsamples of rice from the second series. Thus two composite samples were prepared from each geographical area.

A composite from each of the series of samples was prepared to represent the rice collected within each of the four rice-producing states. The composites were prepared in the following manner: Each 1-lb. sample from a given geographical area was quartered, and a quarter taken for further sampling. This quarter was quartered, a quarter taken, and this in turn was also quartered. This one sixty-fourth of the sample was then combined with a corresponding one sixty-fourth of each of the other two samples from that same area. These three combined sixty-fourths were thoroughly mixed, and then they were combined with corresponding composites from each of the geographical areas from that same state. This state composite was thoroughly mixed and quartered. A portion from each quarter was spread across cross-section paper containing 100 squares, and four kernels of rice were taken from each square. Four such composites were prepared from the first series of samples, and a corresponding four composites were prepared from the second series of 1-lb. samples.

In the preparation of a sample representing the entire rice crop of the Southern region, the composite from each state was quartered, and portions were taken from each quarter in such a manner that the total quantity of rice taken for further sampling corresponded to the number of original 1-lb. samples from which the state composites were prepared. Thus, there were twelve 1-lb. samples in each series from Louisiana, but only nine from Texas. Therefore, in the final sample, there were twelve parts of Louisiana rice to nine parts of Texas rice. Two such composites were prepared, one for each of the series of samples collected. The specimens from each state were combined, as indicated, and thoroughly mixed, and then quartered. A portion from each was spread over cross-section paper, and four kernels were taken from each square.

The variety Zenith made up two of the samples, grade U.S. No. 5. These were combined in the manner indicated above for the composites from the separate areas. These were "reasonably well milled." The "reasonably well-milled" varieties Bluebonnet and Century Patna were also combined in the same manner. The well-milled variety Zenith, grade U.S. No. 5, was examined separately.

Determination of the Microflora

In each instance the kernels were transferred to a sterile Erlenmeyer flask and stoppered with a sterile cotton plug. Water was used for the purpose of removing surface-contaminating microorganisms in the first determinations; the several samples were washed aseptically with ten successive volumes of 75 ml. of sterile distilled water. The flasks were shaken with the water for about 20 seconds at about 200 revolutions

TABLE I

MICROFLORA ON WATER-WASHED MILLED RICE[a]

SAMPLE NO.	AREA AND STATE	VARIETY	STERILE KERNELS	KERNELS CARRYING MOLDS	KERNELS CARRYING BACTERIA AND YEASTS
			%	%	%
ER- 1	New Orleans, La.	Rexoro Zenith Pearl	68.0	1.0	31.0
ER- 2	Beaumont, Tex.	Rexoro Patna BlueBonnet	77.4	1.3	21.3
ER- 3	Lake Charles, La.	Zenith BlueBonnet Rexoro	93.7	1.7	4.6
ER- 4	Crowley, La.	BlueBonnet Zenith	90.7	4.7	4.7
ER- 5	Abbeville, La.	Rexoro BlueBonnet Zenith	96.0	0.7	3.3
ER- 6	El Campo, Tex.	Century Patna BlueBonnet	97.0	0.7	2.3
ER- 7	Dewitt, Ark.	Zenith	88.3	2.3	9.3
ER- 8	Jonesboro, Ark.	Patna Zenith Pearl	87.0	1.3	11.7
ER- 9	Stuttgart, Ark.	Patna BlueBonnet Blue Rose	45.8	5.7	48.3
ER-10	Greenville, Miss.	Century Patna Blue-Bonnet Zenith	91.6	0.7	7.7
ER-11	Houston, Tex.	BlueBonnet Rexoro Zenith	73.3	4.3	22.3
ER-12	Stuttgart, Ark.	Zenith	88.7	3.7	7.6
ER-13	Dewitt, Ark.	Century Patna Zenith Blue Rose	81.7	6.3	12.0
ER-14	New Orleans, La.	Zenith	82.0	6.0	12.0
ER-15	Crowley, La.	Zenith Rexoro BlueBonnet	93.0	3.0	4.0
ER-16	Lake Charles, La.	Patna Magnolia Zenith	91.0	2.7	6.3
ER-17	Greenville, Miss.	Zenith	87.6	4.4	8.0
ER-18	Beaumont, Tex.	BlueBonnet Patna	96.0	2.0	2.0
ER-19	Abbeville, La.	Zenith BlueBonnet	81.0	10.0	9.0
ER-20	Jonesboro, Ark.	BlueBonnet Blue Rose	75.7	4.7	19.7
ER-21	El Campo, Tex.	BlueBonnet	93.0	0.7	6.3
ER-22	Houston, Tex.	BlueBonnet Patna	91.0	0.0	9.0

[a] Actinomycetes, none.

per minute, and the water was drained from each washing. After the last washing, the rice was transferred aseptically to sterile Petri dishes, and individual kernels were picked up with flamed forceps and dropped into Czapek medium (containing 1 g. of sodium nitrate per liter of medium) in 4-in. test tubes. The tubes of medium (autoclaved at 120° for 15 minutes) were supported in racks, with rectangular covers which were lined with cotton pads. The tubes were not plugged individually. The cultures were incubated for 7 days at 25–27°C.

Three hundred kernels were used in the determinations for the individual geographical areas, while 400 kernels were used for each state composite and for each Southern region composite. Three hundred kernels were also used for the U.S. grade No. 5 samples from the Crowley area. Data are recorded in Tables I, II, III, and IV. It will be noted that an average of 97% of the kernels carried no fungi.

Water is not a good de-soiling agent. There is no basis whatever for the assumption that washing with water will remove all surface-contaminating microorganisms. The determinations with the water-washed

TABLE II

MICROFLORA ON MILLED RICE WASHED WITH STERILE WATER ONLY

STATE AND COMPOSITES	STERILE GRAINS	CONTAMINATED GRAINS		
		Mold	Bacteria and Yeasts	Actinomycetes
	%	%	%	%
Louisiana				
ME-1	88.6	2.3	9.0	0.0
ME-6	82.3	5.7	11.3	0.7
Texas				
ME-7	86.3	1.7	12.0	0.0
ME-2	95.3	0.7	4.0	0.0
Arkansas				
ME-3	15.7	3.3	81.0	0.0
ME-8	84.7	1.3	13.7	0.3
Mississippi				
ME-4	94.7	0.3	5.0	0.0
ME-9	89.0	1.7	9.3	0.0

TABLE III

MICROFLORA ON COMPOSITES OF MILLED RICE FROM THE SOUTHERN AREA — WATER-WASHED ONLY

SAMPLE	STERILE GRAINS	MOLDY GRAINS	GRAINS CARRYING BACTERIA	GRAINS CARRYING ACTINOMYCETES
	%	%	%	%
Composite series 1	80.3	2.3	17.3	0.0
Composite series 2	92.5	2.5	4.8	0.3

TABLE IV

MICROFLORA ON WATER-WASHED MILLED RICE, GRADE U.S. NO. 5 (Crowley, Louisiana, Area)

SAMPLE NO.	VARIETY AND MILLING	STERILE KERNELS	KERNELS WITH BACTERIA AND YEASTS	KERNELS WITH ACTINOMYCETES	KERNELS WITH MOLDS
		%	%	%	%
ER-23	Zenith, reasonably well milled	54.0	35.3	0.3	10.6
ER-24	BlueBonnet, Century Patna, reasonably well milled	86.6	10.3	0.0	3.0
ER-25	Zenith, well milled	86.6	12.3	0.0	1.0

samples were carried out for comparison with determinations reported by others who used water for the removal of surface-contaminating organisms (2, 4).

A nonfungicidal and nonbactericidal detergent (Tween-80[5]) was added to the water in two determinations. A portion of the worst sample, ME-3, was washed with 10 successive volumes of 75 ml. of sterile distilled water containing 0.05% and 0.10% of the detergent, following the procedure outlined above. Four hundred kernels were then transferred aseptically and individually to the Czapek medium, and they were incubated for 7 days. Data are recorded in Table V. The number of kernels contaminated with bacteria and yeasts was reduced from 84.7 to 12.9 through the use of the detergent.

TABLE V

RELATIVE EFFECTIVENESS OF WATER, WATER PLUS SURFACE-ACTIVE AGENT, AND SILVER NITRATE FOR DECONTAMINATING SURFACES OF RICE GRAINS

MICROORGANISMS CARRIED	RICE KERNELS CARRYING MICROORGANISMS AFTER TREATMENT WITH			
	Silver Nitrate, 0.1%	Aqueous Tween-80, 0.1%	Aqueous Tween-80, 0.05%	Sterile Water
	%	%	%	%
Sterile	95.0	77.7	67.1	6.3
Fungi	5.0	9.3	10.9	9.0
Bacteria and yeasts	0.0	12.9	21.9	84.7
Actinomycetes	0.0	0.0	0.0	0.0
Penicillium species	0.0	0.0	0.0	0.0
Aspergillus species	0.0	0.5	0.0	0.0

The total number of kernels carrying fungi was not reduced by this treatment. It is noteworthy, however, that no *Aspergillus* species were

[5] Trade names are given as part of the exact experimental conditions and not as an endorsement of the products over those of other manufacturers.

found in the control samples (water-washed) or in the samples washed with the water containing 0.05% of the detergent. Two cultures of *Aspergillus* were found in the samples washed with the water containing 0.10% of the detergent. Thus, only two out of 1200 kernels (0.17%) in the worst lot carried Aspergilli. No Penicillia were found in these samples.

Silver nitrate was used for the purpose of surface-sterilization in several determinations, and the results obtained with this reagent and with the detergent and with water alone are compared in Tables V and VI. The kernels were placed in a 0.1% solution of silver nitrate for 3 minutes, and then the excess solution was drained away. They were then washed in a 0.1% solution of sterile sodium chloride for 3 minutes, and this washing was followed by five successive washings with volumes of 75 ml. of sterile distilled water. Three hundred kernels were planted on Czapek agar medium in the manner noted above.

TABLE VI

COMPARISON OF THE EFFECTIVENESS OF SILVER NITRATE AND OF WATER IN REMOVING SURFACE-CONTAMINATING MICROORGANISMS

SAMPLE NO. AND TREATMENT	STERILE KERNELS	KERNELS WITH FUNGI	KERNELS WITH BACTERIA AND YEASTS	KERNELS WITH ACTINOMYCETES
	%	%	%	%
ER-1 – 0.1% silver nitrate	99.7	0.3	0.0	0.0
Sterile water	93.3	2.7	4.0	0.0
ER-9 – 0.1% silver nitrate	99.3	0.7	0.0	0.0
Sterile water	71.0	4.7	24.3	0.0

None of the kernels treated with silver nitrate carried bacteria. The number of kernels that carried no fungi increased from 91% to 95% in the instance of sample ME-3. The fungi found in the samples of silver nitrate-washed kernels were seven cultures of *Alternaria* species, one of *Phyllosticta* species, four of *Cladosporium* species, and one of *Epicoccum* species. No *Aspergillus* or *Penicillium* species were found. Only 0.3% of the kernels in sample ER-1 which were washed with silver nitrate carried fungi; this was a single culture of an *Epicoccum* species. One culture of a *Helminthosporium* species and one of a *Penicillium* species were found in sample ER-9 that had been washed with the silver nitrate solution. The data recorded in Tables IV and V show that 0.1% of the kernels studied were contaminated with *Aspergillus* species.

An aqueous silver nitrate solution is effective in surface-sterilizing seeds, provided the concentration is great enough and the treatment is

sufficiently prolonged. According to Crocker and Barton (3), treatment of barley seed with a 5% aqueous solution of silver nitrate for 18 hours destroys all of the surface-contaminating fungi and bacteria, but the treatment is worthless for the destruction of pathogenic organisms located in the interior of the seed. The viability of the seed is not affected by the treatment, provided the seeds are washed, after treatment, with a dilute aqueous solution of sodium chloride.

The treatment of the rice samples considered in this communication was mild in comparison with the treatment referred to by Crocker and Barton; the kernels were exposed to a 0.1% solution for 3 minutes, and then they were washed with water (five successive volumes of 100 ml. for 300 kernels) or with dilute aqueous sodium chloride (0.1%), followed by water.[6] It is highly probable, therefore, that the microorganisms in the interior of the kernels were not affected by the treatment.

On this basis, therefore, the 66 samples of rice grown in the Southern Area of the United States, commercially milled and moving in commercial channels, were free from internal infection by bacteria, yeasts, and actinomycetes, and they were virtually free from internal infection by fungi.[7] The percentage of all the surface-sterilized kernels that were free from contamination was 99.1%.

Ninety seven percent of the kernels which were water-washed only for the removal of surface-contaminating microorganisms were free from fungal contamination.

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[6] Only 60% of the kernels in sample ME-3 were found to be sterile when an aliquot was treated for 1 minute with the silver nitrate solution; 96% were sterile after treatment for 2 minutes, and 97% were sterile after treatment for 3 minutes. Apparently 3 minutes is an adequate exposure time for the destruction of the majority of the surface contaminants.

[7] A comparable survey conducted on short-grain rice grown in California the same year showed a similar low microbial population and none that would preclude use of the grain.

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TREATMENT OF WHEAT WITH IONIZING RADIATIONS

I. Some Effects of X-Rays on Gluten and Gluten Sols[1]

Norman E. Lloyd,[2] Max Milner,[2] and K. F. Finney[2, 3]

ABSTRACT

The effect of X-rays on lyophilized wheat gluten under diverse environmental conditions was studied viscometrically. A linear relation existed between the effects of radiation doses administered to dry gluten and the viscosity of 1% sols made from it, while a nonlinear function existed for the relation of dose to viscosity of irradiated 1% sols, indicating that separate mechanisms were responsible for the effects produced in each case. The viscosity losses in gluten sols due to irradiation were practically independent of the temperature within the range of 0° to 50°C. X-rays were more effective in reducing the viscosity of gluten sols at greater dilutions. This supports the activated solvent theory which holds that part of the fraction of energy absorbed by the solvent is transferred to the solute by a mechanism involving free radicals. Hydrogen peroxide produced by X-radiation of aqueous solutions with dissolved oxygen present was shown to play no part in the mechanism responsible for X-ray-induced reduction in viscosity of gluten sols. Sulfhydryl-containing reducing agents showed no protection against radiation-induced reduction in viscosity of gluten sols, as has been reported for other proteins.

Since X-irradiation provides a convenient means of introducing considerable energy into a sensitive biological material such as gluten without appreciably affecting the temperature, it was deemed of interest to explore the effects of such treatment on the molecular structure and chemical properties of gluten. Also it has been proposed recently

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[2] Department of Flour and Feed Milling Industries, Kansas State College, Manhattan.
[3] Chemist, Hard Winter Wheat Quality Laboratory, Field Crops Research Branch, Agricultural Research Service, U.S. Department of Agriculture, Kansas Agricultural Experiment Station, Manhattan.

that ionizing radiations may prevent deterioration of wheat by insects and other biological agents. Studies of this kind with flour have been reported recently by Brownell *et al.* (1). In the present study a viscometric technique was employed to evaluate changes produced by irradiation of partially purified lactic acid dispersions of crude wheat gluten.

Two mechanisms have been postulated to explain the observed chemical and physical changes produced in a substance by ionizing radiations. One is termed the "direct-hit" theory which holds that ions produced in a substance by the direct absorption of radiation cause reactions (9). The other mechanism, known as the "activated-solvent" theory, postulates that part of the energy absorbed by the solvent is transferred to the solute by a mechanism involving hydrogen and hydroxyl-free radicals (8). Irradiation of water apparently produces these free radicals which have great chemical reactivity. Similarly the incorporation of oxygen into aqueous solutions irradiated with X-rays results in the production of hydrogen peroxide, which is postulated to be partly responsible for the lethal effects of X-rays on organisms.

In this present study, the viscosity behavior of gluten and gluten sols when treated with X-rays under diverse conditions involving temperature, protein concentration, and various atmospheres was investigated. The influence on this behavior of certain reducing agents which have a marked effect on gluten viscosity was compared with their effects on other proteins, as has been reported in literature.

Materials and Methods

Lyophilized gluten was prepared by dissolving freshly washed gluten in 0.1 *N* lactic acid, purifying the sol by centrifugation at high velocity, and lyophilizing the purified frozen gluten sol. Seven dry gluten preparations were made from a single flour blended from material representing bread wheats from every major wheat-producing area in the United States. These seven samples were composited for use in the present study. The composite contained 88.00% protein ($N \times 5.7$) on a dry weight basis.

Viscosities were determined at 30 ± 0.04°C. upon lactic acid dispersions containing 1% protein ($N \times 5.7$) at pH 3.2 using modified Ostwald pipets, water-jacketed to maintain constant temperatures, with certain indicated exceptions. At least two determinations were made in every instance and the average was used.

A Picker low-voltage industrial X-ray unit, equipped with a Machlett AEG-50-A tube, was employed to provide high-intensity, long-wavelength X-radiation. The output of the tube was calibrated at 45 kilo-

volts peak and 20 milliamperes by the chemical method of dosimetry of Day and Stein (2). This procedure is based on the production of free radicals in water by ionizing radiation which react quantitatively with aromatic compounds to produce phenols. The latter were assayed colorimetrically. The average of three measurements using a 25-ml. sample placed 2⅜ inches from the target showed that the dose rate was 7120 roentgens per minute. Total dosages administered were controlled subsequently by varying the period of irradiation. It was necessary that the same conditions of sample size, shape, and relative position with respect to the target be maintained for all samples, since the method of dosimetry described was designed to integrate the energy absorbed within a volume defined by these geometric factors. Accordingly, all samples of gluten sols (25 ml.) or dry lyophilized gluten (0.7 g.) were irradiated in the same glass weighing dish (2½ cm. deep and 5 cm. in diameter) which was held in precisely the same position in relation to the target of the X-ray tube by a water-tight adapter made of brass. The fluid samples were kept at 0°C. during irradiation by immersion of the adapter in an ice bath in order to minimize viscosity losses due to traces of proteases present (4).

Samples of gluten and lactic acid gluten sols were irradiated with varying doses of X-rays and their viscosities determined immediately afterward. Samples of the lyophilized gluten weighing 0.7 g. were irradiated at room temperature and were then dissolved to produce 1% sols for the determination of viscosity. Also, 1% gluten sols were irradiated at 0°C. in 25-ml. aliquots.

Results and Discussion

Effect of X-ray Dosage. Dry gluten and gluten sols were treated with X-radiation dosages as high as 700,000 rep.[4]

Figure 1 shows that the viscosity of the sols made up from the irradiated dry gluten decreased linearly as the radiation dosage was increased, whereas the decrease in viscosity of the irradiated sols with increasing dosage was a nonlinear function. The decrease in viscosity indicates that the protein molecules were broken into shorter and/or more symmetrical particles. The greater decrease in viscosity of the irradiated sols suggests that a more efficient mechanism was responsible for the degradation of the protein molecules in solution than when in the dry gluten form.

Influence of Temperature During Irradiation. One percent lactic acid dispersions of gluten were irradiated at varying temperatures con-

[4] One rep (roentgen equivalent physical) = 83 ergs per g. of material treated.

trolled by a constant-temperature water bath and in one instance by a melting ice bath. All sols received a dose of 200,000 rep, following

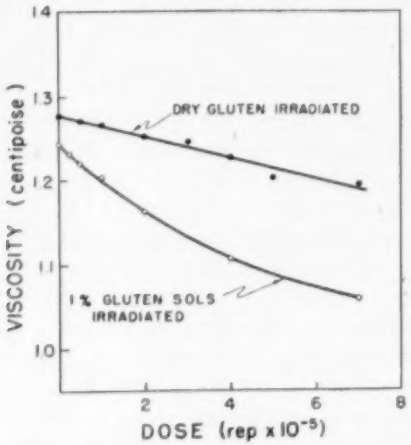

Fig. 1. Change in viscosity of 1% gluten sols in lactic acid due to various irradiation levels.

which viscosities were determined at 30°C. Except for irradiation, non-irradiated controls received the same treatment as the irradiated samples. A plot of the temperature against viscosity in Fig. 2 shows that the temperature coefficient of the X-ray degradation process was noticeable but practically insignificant. All of the slight decrease in viscosity observed in the control sample was attributed to proteolytic activity,

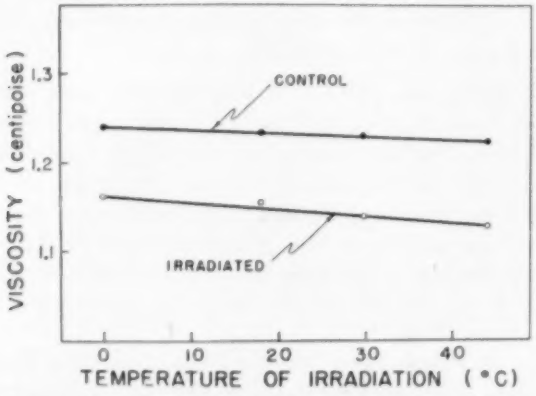

Fig. 2. Influence of temperature during irradiation on X-ray-induced reduction in viscosity of 1% gluten sols.

which would also account for a greater part of the change in the irradiated material.

Effect of Gluten Concentration. Gluten sols were made up at protein concentrations ranging from 1 to 5%. These were irradiated with 200,000 rep at 0°C. after which they were diluted to 1% and their viscosities determined. Nonirradiated controls received identical treatment. The viscosities of the irradiated sols and the controls are plotted in Fig. 3 against the concentrations at which they were irradiated.

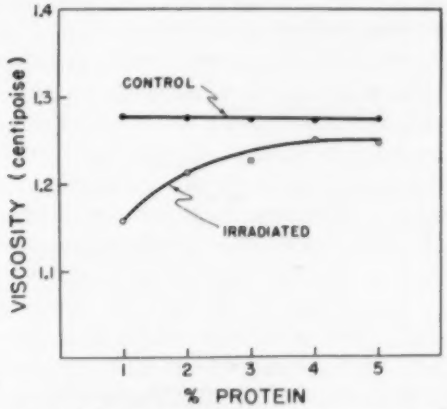

Fig. 3. Influence of gluten concentration on sensitivity to radiation-induced viscosity reduction. Viscosity determined after dilution to 1% protein.

These data show that gluten sols are considerably more sensitive to X-radiation at greater dilutions. This result supports the "activated solvent" hypothesis concerning the effect of ionizing radiations on aqueous solutions.

Influence of Dissolved Oxygen. Risse (7) showed that X-rays produce hydrogen peroxide in water only when dissolved oxygen is present. To determine the effects of radiation-produced hydrogen peroxide on dissolved gluten, 1% sols were prepared by dissolving the lyophilized gluten in nitrogen-air- or oxygen-saturated water under the respective atmospheres. Twenty-five-milliliter aliquots of each preparation were placed in the radiation sample holder fitted with a plastic cover under the same atmosphere and the cover sealed with stopcock grease. Each was administered a dose of 200,000 rep. Nonirradiated controls were treated simultaneously.

The results in Table I show that the radiation-induced decrease in viscosity was at least as great under nitrogen and air as under oxygen.

TABLE I

INFLUENCE OF X-RADIATION (200,000 REP) ON THE VISCOSITY OF GLUTEN SOLS SATURATED WITH NITROGEN, AIR, AND OXYGEN

TREATMENT	VISCOSITY (CENTIPOISES)		
	Control	Irradiated	Decrease Due to Irradiation
Saturated with N_2	1.27	1.16	0.11
Air-saturated	1.24	1.16	0.08
Saturated with O_2	1.28	1.20	0.08

Since elimination of oxygen would preclude hydrogen peroxide formation, these results indicate that any hydrogen peroxide formed was not involved in the mechanism responsible for the reduction of viscosity.

Influence of Reducing Agents. It has been reported that reducing agents have a mitigating influence on the effects of ionizing radiations on proteins. Feinstein (3) studied the protective effects of cysteine, glutathione, and other substances on the viscosity of nucleoprotein sols treated with ionizing radiations. Such reducing agents added before irradiation largely prevented the expected reduction in viscosity, whereas those added immediately after irradiation did not result in such a mitigating effect. Small amounts of reducing agents cause a marked slackening of bread dough and gluten (4, 6). This phenomenon is attributed to direct action of the reducing agent on the gluten protein. The present investigation was undertaken to ascertain if a protective effect would be produced in gluten sols.

TABLE II

VISCOSITIES OF IRRADIATED AND NONIRRADIATED 1% GLUTEN SOLS INCUBATED AT 30°C.

TREATMENT	VISCOSITY (CENTIPOISES)		
	Immediately after Irradiation	2 Hours after Irradiation	10 Hours after Irradiation
Nonirradiated, no reducing agent	1.23	1.21	1.18
Irradiated, no reducing agent	1.17	1.16	1.13
Cysteine added before irradiation	1.00	1.00	0.99
Nonirradiated control	1.01	1.00	0.99
Cysteine added after irradiation	0.99	0.96	0.98
Nonirradiated control	1.01	1.00	0.99
Glutathione added before irradiation	1.13	1.06	1.02
Nonirradiated control	1.18	1.08	1.02
Glutathione added after irradiation	1.13	1.06	1.02
Nonirradiated control	1.19	1.10	1.02
Mercaptoethanol added before irradiation	1.15	1.05	1.00
Nonirradiated control	1.22	1.10	1.02
Mercaptoethanol added after irradiation	1.18	1.08	1.00
Nonirradiated control	1.24	1.10	1.01

Gluten sols, to which cysteine hydrochloride, glutathione, and 2-mercaptoethanol had been added prior to irradiation to make the solutions 0.05 molal, were irradiated with 200,000 rep. The viscosities of the sols were determined immediately afterward and at 2 and 10 hours following irradiation. The sols were incubated at 30°C. between viscosity determinations. Another series of 1% sols were given the same treatment except that the reducing agent was added immediately before the first viscosity determination.

The results presented in Table II show that no protective effect resulted from the addition of reducing agents to the gluten sols either before or after irradiation. Thus, for example, cysteine hydrochloride caused an immediate and drastic reduction of viscosity to approximately 1.00 centipoise, a value all of the other sols treated with reducing agent attained or approached during the 10-hour incubation period.

Discussion

The relation between the amount of X-radiation administered to dry gluten and the viscosity of sols made from such irradiated material was shown to be linear, whereas the effect of irradiation dosages administered to sols of gluten was nonlinear. These results indicate that different interaction mechanisms are involved. Since most of the radiation administered to the aqueous solutions of gluten was absorbed by water molecules, it must be concluded that some of this energy is transferred to the dispersed protein, inasmuch as irradiation by this method was more efficient in reducing viscosity than was irradiation absorbed only by the dry protein.

Irradiation of gluten sols at varying concentrations showed that the protein degradation increased with increasing dilutions of the solution. Assuming the concentration of water molecules activated by the fraction of ionizing radiation absorbed to be constant over the time irradiated and constant at the different concentrations of solute used, the ratio of activated molecules to total solute molecules is greater at the lower concentrations of solute. Therefore it would be expected that a greater percentage of protein molecules would be altered by irradiation in the more dilute solutions, with a resultant greater decrease in viscosity. This phenomenon also supports the "activated-solvent" hypothesis of the action of ionizing radiations on aqueous solutions.

No protection from irradiation was afforded to gluten sols by sulfhydryl-containing reagents, as has been reported for other proteins and organisms (3, 5). On the contrary, the added reducing agents caused greater reductions in viscosity than did the X-radiation. The viscosities of all of the sols were reduced by such agents to values between 0.98

and 1.02 centipoises within a 10-hour period. This indicates that the gluten was affected in the same manner by all three reducing agents, owing possibly to the sulfhydryl group common to all of the reagents.

The present results suggest that irradiation treatment of wheat and flour at dosage rates used in this study can be expected to have significant effects on the physical properties and behavior of gluten during the dough mixing and fermentation processes.

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STUDIES ON CORN PROTEINS
I. A New Method of Extraction[1]

EDWIN T. MERTZ[2] AND RICARDO BRESSANI[3]

ABSTRACT

The proteins of corn germ and corn endosperm can be rapidly and almost completely extracted by an alkaline medium containing sodium, copper, sulfate, and sulfite ions. The ions are removed by dialysis or isoelectric precipitation. In this method the residual nitrogen insoluble in alkali is dissolved by the joint action of copper and sulfite ions.

The extraction of corn proteins presents a special problem because of the anatomical distribution of the endosperm proteins (14) and their insolubility in neutral aqueous solvents. Various extraction methods have been employed (2, 4, 10, 11, 12, 13), but none permits the rapid extraction of the bulk of the proteins. Nearly quantitative extraction of the proteins in corn meal was obtained in 8 days by Foster *et al.* (5) using the commercial anionic detergent, Santomerse No. 3, at high pH; unfortunately, the extracted proteins are bound to the detergent and cannot be separated completely from the latter.

The method in this paper permits rapid extraction of the proteins of both germ and corn endosperm in almost quantitative yields. The protein extracts are relatively free of nonprotein material and are suitable for fractionation and electrophoretic studies.

Materials and Methods

The dry corn kernel is divided into three anatomical parts – seed coat, germ, and endosperm – by soaking the grain in cold water for 3 hours, peeling off the seed coat with tweezers, and dissecting out the germ with a knife. The germ and endosperm fractions are air-dried, finely ground to a particle size of 0.001 inch in a burr mill, and extracted in a Soxhlet for 36 hours with hexane (b.p. 65–67°).

To one part by weight of the defatted germ or endosperm, two parts of distilled water are added, then 0.2% sodium hydroxide solution is added with stirring to approximately pH 9. The slurry is frozen and thawed three times to increase the solubility of the proteins.[4] The

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[2] Associate Professor of Biochemistry.

[3] Rockefeller Foundation Fellow, Department of Biochemistry.

[4] In parallel extractions of the same endosperm sample, omission of the freeze-thaw steps decreased total yield of soluble nitrogen from 92% to 86%.

thawed sample is diluted with 25 parts of distilled water for every one part of original dry sample used. The diluted slurry is then treated with 15 parts of copper sulfate pentahydrate[5] and two parts of sodium sulfite for every 100 parts of original dry sample, and the mixture is stirred to dissolve the added salts. After the salts have been dissolved, the slurry is adjusted slowly to pH 12 by adding concentrated (40%) sodium hydroxide solution to pH 10, and then 0.5*N* sodium hydroxide solution with continuous stirring (alkali zones should be avoided, for they cause gelling, with lowered yields).

The slurry (pH 12) is stirred for 3 hours at 25°C., then centrifuged at 2000 r.p.m. for about 20 minutes. The supernatant is saved, and the residue is stirred for 1 hour at 25°C., with 10 parts of sodium hydroxide solution (pH 12) for every one part of original dry corn fraction. The slurry is centrifuged, and the supernatant "washing" combined with the supernatant from the first extraction. The combined extract is frozen, thawed, and centrifuged to remove precipitated starch. The supernatant is dialyzed against two changes per day of 4 liters of 0.02*N* hydrochloric acid solution per 120 mg. of corn nitrogen, for 3 days at 0°C., then for 3 days against distilled water at 0°C. The almost copper-free, salt-free suspension of corn proteins[6] is pre-evaporated to about one-fourth its original volume and lyophilized. The dry product is a fluffy powder, total nitrogen content 11–12%, which is readily soluble in carbonate-bicarbonate buffer (pH 11) and in sodium hydroxide solution (pH 12), and soluble to the extent of approximately 26% in water and 44% in 70% ethanol. About 90% of the total nitrogen of the extract is recovered as dry (lyophilized) product. The remaining 10% passes through the dialysis membrane.

The same recovery of corn protein can be obtained by omitting the dialysis step, and precipitating the proteins directly by adjustment of the copper sulfate solution to pH 6.0 (endosperm proteins) or to pH 5.5 (germ proteins). These are the points of minimum solubility as determined from pH precipitation curves. At these points about 10% of the nitrogen of the extract is again soluble.

The data reported in this paper were obtained on a sample of commercial hybrid yellow corn seed (1.31% total nitrogen) which yielded on a weight basis 6.3% seed coat, 7.4% germ, and 86.3% endosperm. These seed parts contained 0.57%, 2.56%, and 1.15% total nitrogen, respectively. The lipid-free germ and endosperm contained 3.87% and 1.23% nitrogen, respectively.

[5] We are indebted to Dr. E. L. Powell, American Maize Products Company, Roby, Indiana, for suggesting the use of copper sulfate as a solubilizing agent.

[6] Contains approximately 80 parts of copper per one million parts of solids.

Results

Extraction of Endosperm. Table I shows the effect of changing the standard conditions of extraction. Extraction is almost complete at

TABLE I

EFFECT OF VARIATION ON EXTRACTION OF ENDOSPERM NITROGEN

ITEM NO.	CHANGES FROM STANDARD CONDITIONS[a]	YIELD OF TOTAL NITROGEN
		%
1	No changes	95.3
2	2-hour extraction	89.3
3	6-hour extraction	98.0
4	14-hour extraction	96.7
5	18-hour extraction	95.7
6	0°C.	91.4
7	0°C., alkali only (no copper sulfate or sodium sulfite)	59.8
8	0°C., alkali, two parts sodium sulfite (no copper sulfate)	42.5
9	0°C., five parts copper sulfate, two parts sodium sulfite, alkali	89.9
10	0°C., 10 parts copper sulfate, two parts sodium sulfite, alkali	88.8
11	Two parts sodium sulfite, pH 5.6 (no copper sulfate)	20.3
12	Fifteen parts copper sulfate, alkali (no sodium sulfite)	73.7
13	One part sodium sulfite, fifteen parts copper sulfate, alkali	92.8
14	Three parts sodium sulfite, fifteen parts copper sulfate, alkali	93.4

[a] Standard conditions: 3-hour extraction at 25°C.; pH 12; 15 parts copper sulfate pentahydrate and two parts sodium sulfite per 100 parts original dry endosperm sample.

the end of 2 hours, and is as complete at the end of 3 hours as it is at the end of 18 hours (items 1–5). At low temperature and high pH, with copper ion and sulfite ion present, over 90% of the nitrogen is extracted (item 6). With alkali alone, however, only about 60% of the nitrogen is extracted (item 7). Addition of sulfite ion to the alkali appeared to depress the solubility of the corn proteins (item 8). The addition of 5, 10, or 15 parts of copper sulfate to the alkali sulfite solution doubled the yield of nitrogen extracted (items 9, 10, and 6). At room temperature, sulfite ion alone in slightly acid medium (pH 5.6) extracts only one-fifth of the nitrogen (item 11), whereas copper ion alone at high pH extracts about three-fourths of the nitrogen (item 12). Extraction of the endosperm sample first with sulfite at low pH (item 11), and then with the standard amount of copper sulfate at high pH (same as item 12 but data not shown in Table I) gave a recovery ($20.3\% + 72.5\% = 92.8\%$), which is almost as high as that obtained with all reagents present simultaneously (item 1). Conversely, extraction of endosperm directly with copper sulfate and alkali in the absence of sodium sulfite gave a yield of 73.7% (item 12) which agrees with that obtained from endosperm (72.5%) previously relieved of one-fifth of its nitrogen by acid sulfite (pH 5.6). It is thus apparent that copper sulfate solubilizes a fraction of the nitrogen not soluble in

alkali alone or in sulfite ion alone, or in a combination of alkali and sulfite. In turn, sulfite ion solubilizes a fraction of the nitrogen which is not soluble in the combination of alkali and copper ion alone (compare item 12 with items 13, 1, and 14). The key to the solubilization of nitrogen in the corn endosperm by this method is not the presence of a single agent, but rather the joint action of alkali, copper ion, and sulfite ion.[7]

TABLE II

EFFECT OF VARIATIONS ON EXTRACTION OF GERM NITROGEN

ITEM NO.	CHANGES FROM STANDARD CONDITIONS[a]	YIELD OF TOTAL NITROGEN
		%
1	No changes	97.9
2	No copper sulfate, no sodium sulfite, 0°C.	70.1
3	No copper sulfate, 0°C.	69.7
4	No sodium sulfite, 0°C.	66.6
5	0°C.	97.1

[a] Standard conditions: Same as in Table I.

Extraction of Germ. The data in Table II show that the nitrogen of corn germ also can be extracted nearly quantitatively with the copper method (item 1). About two-thirds of the total nitrogen can be extracted with alkali alone (item 2), and the separate addition of either

TABLE III

EXTRACTION OF THE SAME ENDOSPERM BY OTHER METHODS

MODIFIED OSBORNE METHOD[a]	EXTRACTION TIME	YIELD OF TOTAL NITROGEN
	hrs.	%
1 5% Potassium chloride	2	12.4
2 71% Ethanol	3	33.9
3 0.2% Sodium hydroxide	1	36.8
Total		83.1
Detergent method[b]		
1 Single extraction (0°C.)	3	38.7
2 Single extraction (25°C.)	48	72.7
	96	88.1
	144	92.1
	192	93.8

[a] See reference 10.
[b] See reference 5.

sodium sulfite (item 3) or copper sulfate (item 4) does not increase the yield of nitrogen. However, with a combination of all three reagents, 97% of the nitrogen of the germ is extracted even at 0°C. (item 5).

[7] The alkali-copper-sulfite method is equally effective with commercial corn gluten from the wet milling process, the standard conditions giving yields of 95–98% of the total gluten nitrogen.

Here again the solubilizing effect of copper and sulfite ions appears to depend on joint activity at high pH.

Comparison with Other Methods. Samples of the same endosperm were also extracted using a modified Osborne method (10) and a method based on the solubilizing action of the detergent, Santomerse No. 3 (5). The results of these extractions are shown in Table III.

The Osborne method (2) and its modifications are time-consuming and give low yields, the modification (10) tested on our endosperm sample giving a yield of 83.1% of the total nitrogen.

Using Santomerse No. 3, a single extraction of 3 hours at low temperature yielded 38.7% of the nitrogen (Table III), as compared with a yield of 91.4% with the copper method (see Table I, item 6). At room temperature, the yield of nitrogen at the end of 192 hours was about the same as that obtained with the copper method. The copper extract (item 1, Table I), which had been freed of all but traces of copper ion, and the 192-hour detergent extract (Table III), were dialyzed against bicarbonate buffer, pH 10.85 ($\mu = 0.26$) and analyzed in a Perkin-Elmer Model 38 electrophoresis apparatus. The detergent extract contained at least four electrophoretically distinct components with mobilities ranging from 4.5 to 14.6×10^{-5} cm^2/v.sec. This does not include the fastest component, which is the detergent itself (5). The protein solution obtained by the copper method had at least five electrophoretically distinct components with mobilities ranging from 3.3 to 8.8×10^{-5} cm^2/v.sec. A more extensive report on the electrophoretic properties of endosperm proteins extracted by the copper method will be published at a later date.

Possible Effects of Copper Reagent on the Native Corn Proteins. The use of a reagent containing alkali, sulfite, and cupric ion, all of which are known to alter the protein molecule, is justified in the case of corn endosperm only because of the pronounced insolubility of corn endosperm nitrogen in milder solvents. However, the proteins extracted by this reagent are not unlike those extracted by other methods (2, 4, 10, 11, 12, 13) and will be described in later papers.

Some of the possible unfavorable effects of the high pH used in our procedure are ammonia liberation, cystine destruction, arginine cleavage, and racemization. However, no release of ammonia from corn endosperm treated with copper reagent could be detected in preliminary tests,[8] and the glutelin fractions isolated contain levels of cystine and arginine[8] which are substantially higher than that of either commercial zein or corn gluten (1). In addition, no change in optical rotation of commercial zein dissolved in the copper reagent at room tem-

perature could be detected in a period of 12 hours,[8] suggesting that with zein either racemization is not occurring, or the changes are compensatory. Other endosperm proteins have not been tested.

The primary action of sulfite on proteins is the reduction of the disulfide groups present. If the reaction of peptide-bound cystine is similar to that of free cystine, then the end products from each cystine residue would be one molecule of peptide-bound cysteine and one molecule of peptide-bound salt of S-cysteine sulfonate (3). However, Middlebrook and Phillips (9) observed that the pH optimum for this reaction with wool proteins is 5.0, and that the reaction rate falls off rapidly above and below this pH. It is thus possible that the reducing effect of sulfite is at a minimum under our extraction conditions.

Specific reactions of copper ion with proteins are not well characterized. Klotz (6, 7, 8) has attempted to pin-point the sites at which copper is bound to proteins. On the basis of spectrophotometric data, he concluded that divalent copper is bound primarily to the free acidic (including sulfhydryl) and basic groups of proteins at pH 4 to 8, and that at pH values above 8, the copper is tetracoordinated. At least two of the copper bonds in the latter case are of the amine type. It is probable that the bonding at high pH of copper ions with two to four centers containing nitrogen (peptide, amide, amino, etc.) is in a large measure responsible for the solubilizing effect of copper ions on corn endosperm proteins.

Whether the original specific configurations of the native proteins solubilized by the copper reagent are restored on removal of the copper and sulfite ions at low pH is not known, and requires further study.

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A NOTE ON IMPROVED INTERPRETATION OF THE 2,3,5-TRIPHENYLTETRAZOLIUM CHLORIDE COLOR TEST FOR VIABILITY AS AN INDICATION OF THE PROCESSING VALUE OF CORN[1]

R. U. Schenk, M. M. MacMasters, and F. R. Senti[2]

Earlier work at this laboratory (1) indicated that the color test for viability made with 2,3,5-triphenyltetrazolium chloride (2,3,4) might be useful for distinguishing corn that had been overheated during artificial drying and thus lowered in value for processing. However, some instances were later encountered in which starch yield by laboratory processing was poor, in spite of high viability indicated by the test. Some of these samples were chosen for comparison of the apparent viability by the test with 2,3,5-triphenyltetrazolium chloride reagent and actual germinability, because it was thought that kernels killed by molds might be giving a positive viability test.

Each kernel of samples taken for viability determination was split longitudinally through the germ. The opposite halves were placed, split side up, on sand in two Petri dishes. The kernels in one dish were then flooded with a 1% aqueous solution of 2,3,5-triphenyltetrazolium chloride. This dish was placed in the dark, as the reagent is sensitive to light. The sand in the other dish was moistened with distilled water. Sound, viable corn was similarly treated as a control. The halves treat-

[1] Manuscript received May 4, 1956.

[2] Northern Utilization Research Branch, Agricultural Research Service, U. S. Department of Agriculture, Peoria, Illinois.

ed with 2,3,5-triphenyltetrazolium chloride were examined after 1 hour. The germinating halves were examined at 1 and 2 days after the test was started.

Halves of the control kernels were fully colored by the reagent. The corresponding halves on moist sand showed rapid elongation of the growing parts. In contrast, some halves of kernels of the questionable samples gave the test for viability in the reagent, although their matching halves on moist sand showed no elongation of growing parts. Instead, the germs were rapidly overgrown by molds. Apparently, the positive viability test in these cases indicated viability of molds rather than of the corn. The halves in the reagent that corresponded to the moldy halves were found to have germs that often were discolored and almost invariably were of friable texture. The friable texture became more apparent during the course of treatment with 2,3,5-triphenyltetrazolium chloride. It is known that deteriorative changes resulting in the formation of friable germ reduce the value of the corn for millings, since germ recovery is adversely affected.

Examination of otherwise sound corn known to have been killed by heat treatment showed that the germs were firm and rubbery and did not become colored in the test reagent.

A relatively accurate differentiation can therefore be made between sound, mold-killed, and heat-killed corn by careful examination of the germ texture in conjunction with the 2,3,5-triphenyltetrazolium chloride color test. If mold develops in germ that has died from some other cause, however, the original cause of death cannot be determined by this method. This may be of little concern to the corn processor, since it is generally considered that death of the germ, from whatever cause, lowers the value of the corn for milling.

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The selective or combined use of these heating elements provide seven operating ranges, namely, 150, 250, 350, 400, 500, 600 and 750 watts. The heating elements are energized by three toggle switches located on the control panel. A panel plate clearly indicates which toggle switch must be thrown to obtain the desired wattage.

The entire heating unit is contained in a refractory plate isolated from the outer frame insuring that the stainless steel case is cool at all times.

Removable upper refractory is reversible. One side molded to accommodate 500 ml and 800 ml Kjeldahl flasks and 4 in. concentric metal rings. The other side beveled to accommodate large, round-bottom flasks.

Complete with S-40546 lower refractory, S-40547 upper refractory, one pair of dovetail clamp sockets, one S-40745 dovetail clamp and cord and plug for attachment to standard outlets. For operation from 115 volt, A.C. or D.C. circuits..... **$28.00**

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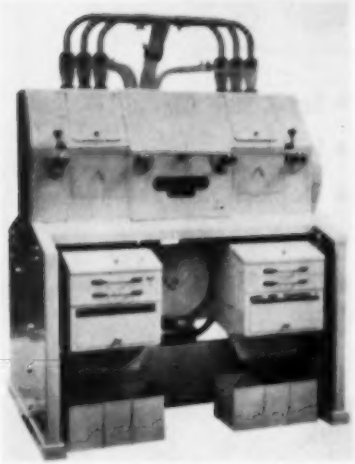
BUHLER

AUTOMATIC LABORATORY MILL



Front side of the MLU 202 Buhler Automatic Laboratory Mill; equipped with pneumatic product conveying which eliminates material collecting in the ducts and resultant infestation.

Offers You These Advantages

—It grinds small samples, or large laboratory runs;

—It produces flour with exactly the same baking quality as the flour from a commercial mill;

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—It grinds wheat quickly and automatically; empties completely;

—It is simple to operate and easy to attend;

—It requires no special milling experience from the operator.

The MLU 202 Laboratory Mill was designed primarily to produce flour from any specific sample of wheat so that advance information may be obtained as to what can be expected in yield, ash content, color and baking qualities of the sampled wheat, without running it through a commercial unit. The Buhler MLU 202 has the same advantages as a pneumatic mill in comparison with an elevator mill.

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